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Complete List of Authors:	Dietl, Alexander; University Hospital Regensburg, Department of Internal Medicine II Zimmermann, Martina; University of Regensburg, Department of Genetic Epidemiology Brandl, Caroline; University of Regensburg, Department of Genetic Epidemiology; University Hospital Regensburg, Department of Ophthalmology Wallner, Stefan; University Hospital Regensburg, Institute of Clinical Chemistry and Laboratory Medicine Burkhardt, Ralph; University Hospital Regensburg, Institute of Clinical Chemistry and Laboratory Medicine Maier, Lars; University Hospital Regensburg, Department of Internal Medicine II Luchner, Andreas; Krankenhaus Barmherzige Bruder Regensburg, Department of Cardiology Heid, Iris; University of Regensburg, Department of Genetic Epidemiology Stark, Klaus; University of Regensburg, Department of Genetic Epidemiology
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High-Sensitivity Cardiac Troponin T – reference limits and specificity in the elderly

Alexander Dietl[1], Martina E. Zimmermann[2], Caroline Brandl[2,3], Stefan Wallner[4], Ralph Burkhardt[4], Lars S. Maier[1], Andreas Luchner[5], Iris M. Heid[2], Klaus J. Stark[2]

[1] Department of Internal Medicine II, University Hospital Regensburg, Regensburg, Germany

[2] Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany

[3] Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany

[4] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

[5] Department of Cardiology, Krankenhaus der Barmherzigen Brueder, Regensburg, Germany

Corresponding author:

Dr. Alexander Dietl

University Hospital Regensburg

Department of Internal Medicine II

D-93042 Regensburg

Germany

Email : alexander.dietl@ukr.de

Phone : +49 941 944 0

Fax : +49 941 944 5212

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Abstract

Objective: European guidelines recommended a uniform upper-reference limit of high sensitivity cardiac troponinT (hsTnT) to rule-out non-ST-segment-elevation myocardial infarction. Our study aimed to provide a hsTnT reference-distribution and to assess the specificity of the 14ng/l cut-off value in the mobile population ≥70years of age.

Design: A cross-sectional analysis was performed in the German AugUR study (*A*ge-related diseases: *u*nderstanding *g*enetic and non-genetic influences – a study at the *U*niversity of *R*egensburg).

Setting: Study population was the mobile population aged 70+ years living in the city and county of Regensburg, Germany.

Participants: A random sample was derived from the local population registries of residence. Of the 5,644 individuals invited, 1,133 participated (response rate=20.1%). All participants came to the study centre and were mentally and physically mobile to conduct the protocol (face-to-face interview, blood draw, standardized transthoracic echocardiography). None of the participants was in an acute state of myocardial infarction.

Results: Among the 1,129 individuals with hsTnT measurements (overall median=10.0ng/l, interquartile range [IQR]=8.0), hsTnT was higher among the older individuals and higher among men (men 70-74years median=9.6ng/l, IQR=5.9ng/l; men 90-95years median=21.2ng/l IQR=11.4ng/l; women 70-74 years median=6.3ng/l, IQR=4.0ng/l; women 90-95years median=18.0ng/l, IQR=10.0ng/l). In participants with impaired kidney function ($eGFR_{crea}$<60ml/min/1.73m^2), hsTnT was elevated (median=13.6ng/l, IQR=11.2ng/l). Specificity of recommended upper-reference limit, 14ng/l, is 68%. Most false positives were among men aged >79years (specificity=34%). In a healthy subgroup (n=106, none of the following: overt heart disease, impaired renal function, left ventricular hypertrophy, diastolic/systolic dysfunction), specificity was 91%. The 99th percentile of the healthy subgroup was twice as high as the recommended cut-off value (29ng/l, 95%-confidence interval=20-29ng/l).

Conclusion: In the elderly population without acute myocardial infarction, hsTnT further increases with age showing different levels for men and women. The specificity of the 14ng/l cut-off is considerably lower than 99%, even in healthy subjects.

Article Summary

Strengths and limitations of this study

A major strength of our study is the population-based approach focussed on the age group, which is most often seen in chest pain units.

The study was a-priori designed to determine reference values of biomarker incorporating thorough protocols for collection of serum and elaborated biobanking.

The study protocol entailed firmly standardised procedures as well as the conduct by trained, experienced and quality-controlled staff.

Echocardiography was performed according to current European and American guidelines following in advance defined standard operating procedures.

As the recruitment area in South-Eastern Germany implies a largely Caucasian population, we cannot report on high-sensitivity troponinT concentrations in further ethnicities.

Introduction

High sensitivity cardiac troponin T (hsTnT) is a sensitive marker of cardiomyocyte injury indicating myocardial damage resulting from, e.g. myocardial ischaemia, pulmonary embolism, myocarditis or Takotsubo syndrome[1–3]. In chest pain patients, hsTnT constitutes a mainstay for diagnosis of non-ST-segment elevation myocardial infarction. The 2020 Guidelines of the European Society of Cardiology for the management of acute coronary syndromes continue to recommend a uniform cut-off concentration of 14ng/l for rule-out of non-ST-elevation acute myocardial infarction in the 0/2-hour protocol. This hsTnT-value was initially derived from a pooled reference population of 616 subjects (mean age 44 years) and a study sample comprising 533 individuals (mean age 37 years), in which a value of 14ng/l signified approximately the 99th percentile of hsTnT-distribution [4,5]. In several further analyses, it turned out to be a sufficiently sensitive upper reference limit for rule-out of acute myocardial infarction in the emergency department [1,6,7]. However, large population-based studies have challenged uniform cut-off values due to considerable sex- and age-differences in hsTnT-distribution with decreasing specificity by age [8–10]. The dependency of hsTnT-concentrations on age implies major clinical impact: most chest pain patients are at advanced age [11] and the decreasing specificity of the uniform cut-off by age yields a growing number of false-positive results in the elderly [12,13]. Despite being the primary clinical target population for the application of these cut-off values, the elderly are less captured in published data on hsTnT-distribution [8–10]. This gap can be attributed to the specific needs of the elderly, which often hamper their participation in population-based studies or prompt general studies to exclude individuals above the age of, e.g., 70 years[14,15]. The aims of our analyses were to provide a reference distribution for hsTnT in the population ≥70years of age and to quantify the specificity of the 14ng/l cut-off value. We report on our cross-sectional data from 1,129 participants of the German AugUR study (*A*ge-related diseases: *u*nderstanding *g*enetic and non-genetic influences – a study at the *U*niversity of *R*egensburg), which focused on the mobile population ≥70years of age. The study protocol entailed a face-to-face interview, collection of serum samples and a standardized transthoracic echocardiography enabling a thorough assessment of even subtle subclinical cardiac disorders.

Methods

Study sample

The design of the German AugUR study (**A**ge-related diseases: **u**nderstanding **g**enetic and non-genetic influences – a study at the **U**niversity of **R**egensburg) has been described in detail previously[16]. Briefly, we recruited inhabitants at least 70 years of age in the city of Regensburg, Germany, and selected nearby counties via a random sample from the local registries of residence. Participants were invited by mail, had to be willing and able to come to the study centre, to conduct a standardized in-person interview with the study assistant, and to undergo various non-invasive medical exams.

Ethics statement

The study protocol, study procedures, and data protection strategy were all approved by the Ethics Committee of the University of Regensburg, Germany (vote 12-101-0258). All study participants provided written consent after being informed about the study. The study was conducted according to the principles expressed in the Declaration of Helsinki. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

General data assessment

Sociodemographic factors, smoking behaviour, medication use and cardiovascular medical history (including existence, history, and time onset of cardiovascular diseases and interventions) were assessed in a standardised face-to-face interview by trained staff. Blood pressure was measured using an automatic device (Omron M10-IT; Omron Healthcare, Kyoto, Japan), pulse rate was determined by palpation after five minutes of resting time. Blood pressure was measured three times and the average of the second and third measurement were computed for further analyses.

Assessment of cardiac morphology and function by echocardiography

In order to assess even subclinical cardiac disorders, transthoracic echocardiography was performed using a commercially available ultrasound unit (HP Sonos 5500 with 2-4 MHz probe; Philips, Eindhoven, The Netherlands). The stored tracings were evaluated post hoc using analytical software Xcelera R3.2L1 Version 3.2.1.520 – 2011 (Philips Medical Systems, Amsterdam, Netherlands) as previously described[17]. The echocardiographic program focused on left atrial and ventricular morphology and function accounting for chamber-specific cardiac remodelling processes[18] according the current guidelines[19]: left atrial volume was determined by two-dimensional volumetric measurement based on tracings of the blood-tissue interface in apical four-chamber view. M-Mode measurements for calculating left ventricular mass were obtained from parasternal long axis view and determined perpendicular to the left ventricular axis. Left ventricular mass was computed by the Devereux formula[20]. Left atrial volume and left ventricular mass were indexed to body-surface area approximated by DuBois' formula[21]. To estimate left ventricular filling pressures, the ratio of the transmitral early peak velocity by pulsed wave Doppler (E) over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler (mean e') was determined (E/mean e'). Left ventricular diastolic dysfunction was evaluated according to recent recommendations[22]. Systolic function was assessed as ejection fraction estimated by the modified Simpson's method[19] based on monoplanar measurements in the apical four chamber view. Each of the measurements used for further analyses was repeated three times for regular rhythm and ten times in case of arrhythmia to reduce random error.

High-sensitivity Troponin T and NT-proBNP measurements

Collection and procession of biosamples were conducted following standard operation procedures developed for this study based on established methods and recommendations[23]. Deviations from these standard operation procedures (e.g., extended sample handling at room temperature) were recorded and linked with the biosample information. All samples were processed immediately and kept on dry ice before final storage at the end of the day. Identification, assignment and link to electronic case report form (eCRF)

data for biosamples including 2D-barcoded tubes were managed by self-developed integrated software.

Non-fasting blood samples were drawn in a sitting position after at least five minutes of resting. Mild venous stasis was applied for a maximum duration of one minute. Whole blood was taken using a 21G multifly needle. Two samples were used for ad hoc analysis. Serum tubes with clot activator were left in upright position for 30 minutes after blood draw and were centrifuged at 2,000 g for 15 minutes at room temperature to separate serum from the cellular fraction as soon as possible. Supernatants from serum tubes were transferred to 2D-barcoded tubes for storage at -80°C.

Measurements for hsTnT and N-terminal prohormone B-type (brain) natriuretic peptide (NT-proBNP) were conducted in stored serum samples by the Department of Clinical Chemistry and Laboratory Medicine of the University Hospital Regensburg on a cobas e411 (Roche Diagnostics, Rotkreuz, Switzerland). After measurement, data were exported from SWISSLAB (NEXUS SWISSLAB GmbH, Berlin, Germany) in Excel format and processed with Microsoft Access 2019 (Microsoft Corporation, Redmond, WA, USA), SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and SPSS 25.0.0.2 (IBM Corporation, Armonk, NY, USA.). 31 values for hsTnT [ng/l or pg/ml] were on the lower detection limit of "<3" ng/l. Those results were winsorised to "2.9" to discriminate from true "3.0". For NT-proBNP [pg/ml], no values with extremes beyond specified measurement range (5-35,000 pg/ml) were detected.

Statistical methods

Continuous variables are reported as mean and standard deviation (SD) or as median with interquartile range (IQR) and different percentiles. Estimates of confidence intervals for 99th and 95th percentiles were derived by bootstrap analysis using bias corrected and accelerated intervals. Categorical variables are reported as proportions. Odds ratio estimates for hsTnT were computed by simple logistic regression. We used the STROBE cross sectional checklist when writing our report[24]. All analyses were carried out with SPSS 25.0.0.2 (IBM, Armonk, USA).

Results

Characteristics of the study sample

1,129 participants out of 1,133 showed valid hsTnT-values and were included for further analyses. Age ranged from 70.3 to 95.0 years, with a median of 76.7 years (interquartile range [IQR] 7.2 years). Demographic, clinical and laboratory characteristics are shown in **Table 1**. Of note, all individuals came walking to the study centre at the University medical centre, participated in the three-hour study program with little exhaustion mentally or physically and can thus be considered mobile elderly. None of the participants had any sign of acute cardiac condition, particularly myocardial infarction, throughout the study visit. While our participants were all relatively healthy by design, they included medical conditions to the extent as one expects from the mobile population of that age.

Characteristics	Women (n=509)	Men (n=620)
Age [years]	77.34 ± 5.02	77.88 ± 5.06
Body-mass index [kg/m^2]	27.8 ± 5.0	28.2 ± 4.0
Diabetes [%]	19.4	23.2
Hypertension [%]	74.2	72.9
Coronary artery disease [%]	9.8	23.1
Heart failure [%]	16.0	13.5
Tobacco use (present/past) [%]	25.5	60.3
$eGFR_{crea}$ [ml/min/1.73m^2]	68.5 ± 16.2	66.1 ± 16.4

Table 1: Baseline characteristics of the study sample

Shown are mean and standard deviation or proportions for the 1,129 subjects separately for women and men.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Distribution of hsTnT-values by age, sex and glomerular filtration rate

First, we looked at the distribution of hsTnT levels by age groups and sex **(Figure 1)**. HsTnT-values increased with age and were higher in men than in women (**Table 2**). Further, we report on values separately for normal and reduced glomerular filtration rate (eGFR≥ vs. < 60ml/min per 1.73m^2, derived from serum creatinine, **Table 3**).

For actual diagnosis of acute non-ST-elevation myocardial infarction in symptomatic patients, the 2020 Guidelines of the European Society of Cardiology endorse a rule-in hsTnT-cut-off concentration of 52ng/l, which implies immediate referral of chest pain patients to invasive diagnostics[1]. In 13 subjects (1,2%) of our study, hsTnT was measured above this rule-in cut-off (≥ 52ng/l) with a median of 72.1ng/l (IQR 46.9) and a maximum of 421ng/l.

Specificity of the rule-out upper reference limit (14ng/l) in the mobile elderly

Next, we intended to estimate the specificity of the endorsed rule-out upper reference limit of hsTnT [1] in our mobile elderly individuals considered free of acute myocardial infarction **(Figure 2).** Applying the recommended cut-off value of 14ng/l, 70% (790/1,129 subjects) of our study participants were below this cut-off. Main determinants of hsTnT-values above 14ng/l were age, male sex, impaired kidney function, type II diabetes, history of coronary artery disease, left ventricular hypertrophy, diastolic dysfunction, left atrial hypertrophy and elevated filling pressure (E/e'>14, **Figure 3**). As this cut-off was defined as the 99th percentile of reference samples without acute myocardial infarction in the attempt to yield 99% specificity [1,4,5], this is in line with the notion that, among our study participants, 70% were correctly identified (true negative for acute myocardial infarction), but 30% (339/1,129) were not (false-positive). These 339 individuals showed a median level of 19.4ng/l (IQR 9.0). They were older, more likely men and more likely with diabetes, stable coronary artery disease, heart failure or impaired kidney function than the 790 individuals below the cut-off (**Table 4**). Regarding echocardiographic measurements, elevated left ventricular mass was detected.

Further stratification revealed a particularly low specificity for the 14ng/l hsTnT level in men (57%) as well as in subjects with impaired kidney function (50% for eGFR < 60 ml/min/1.73m^2) and bottommost in men aged 80 years or older (34%, **Table 5**).

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
Women n	191	184	89	33	12	509
Mean ± SD	7.54 ± 4.56	9.76 ± 8.51	11.03 ± 6.32	16.66 ± 13.62	17.02 ± 6.97	9.77 ± 7.75
Minimum	2.9	2.9	2.9	6.0	4.9	2.9
5th percentile	2.9	2.9	4.5	6.2	4.9	3.1
10th percentile	3.3	3.6	5.2	6.9	6.4	4.0
25th percentile	4.7	6.1	7.2	9.7	11.0	5.7
Median	6.3	8.2	9.5	13.1	18.0	8.0
75th percentile	8.7	11.3	13.0	18.9	2.10	11.5
90th percentile	13.5	15.1	18.7	27.8	28.7	17.4
95th percentile	16.1	19.7	22.5	55.1	-	21.5
Maximum	32.8	102.8	40.6	78.8	31.3	102.8
Men n	207	226	116	57	14	620
Mean ± SD	11.96 ± 11.49	16.31 ± 28.60	16.71 ± 9.87	21.89 ± 10.31	21.16 ± 8.98	15.56 ± 19.49
Minimum	2.9	2.9	5.2	10.4	8.6	2.9
5th percentile	4.8	5.2	6.6	11.3	8.6	5.3
10th percentile	5.6	6.2	7.5	12.5	9.4	6.1
25th percentile	7.2	8.6	9.6	15.5	14.6	8.3
Median	9.6	12.8	14.5	20.4	21.2	12.3
75th percentile	13.1	18.0	20.3	25.6	26.0	18.1
90th percentile	18.4	24.5	30.2	30.2	36.1	26.4
95th percentile	28.4	30.4	37.7	38.0	-	31.3
Maximum	107.1	421.3	56.5	74.6	44.0	421.3

Table 2: hsTroponinT values [ng/l] by age groups and sex in 1,129 participants of the AugUR stud

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
$eGFR_{crea}$ ≥60 n	322	298	113	35	10	778
Mean ± SD	9.16 ± 7.68	10.93 ± 7.79	12.31 ± 6.42	17.31 ± 7.29	14.91 ± 14.75	10.74 ± 7.74
Minimum	2.9	2.9	2.9	6.3	4.9	2.9
5th percentile	3.1	3.1	5.2	6.5	4.9	3.2
10th percentile	4.0	4.7	5.8	8.3	5.3	4.5
25th percentile	5.5	6.9	7.9	11.1	9.8	6.3
Median	7.4	9.2	10.7	16.8	14.8	8.9
75th percentile	10.4	13.2	15.3	22.4	17.8	13.2
90th percentile	14.7	18.8	21.4	28.6	27.5	18.5
95th percentile	21.0	22.6	24.7	29.7	-	23.5
Maximum	107.1	102.8	40.6	33.1	28.2	107.1
$eGFR_{crea}$ <60 n	70	108	90	54	16	338
Mean ± SD	13.07 ± 13.85	20.38 ± 40.60	16.55 ± 10.98	21.95 ± 13.74	21.96 ± 8.18	18.17 ± 25.25
Minimum	2.9	3.4	4.7	7.3	9.8	2.9
5th percentile	4.1	4.8	5.5	9.9	9.8	5.3
10th percentile	5.3	6.9	6.9	11.8	10.0	6.8
25th percentile	7.0	9.7	9.1	13.5	19.4	9.4
Median	10.0	13.5	12.2	19.0	21.2	13.6
75th percentile	13.7	19.7	20.8	23.7	25.8	20.6
90th percentile	20.8	29.0	32.1	33.7	35.1	29.3
95th percentile	30.2	47.4	41.3	58.3	-	43.6
Maximum	101.8	421.3	56.5	78.8	44.0	421.3

Table 3: hsTroponinT values [ng/l] by age groups and $eGFR_{crea}$ categories in 1,129 participants of the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m²; a value of 60 was used to determine between good and limited kidney function

hsTroponinT [ng/l]	<14ng/l	n	≥ 14 ng/l	n
Age [years]	76.5 ± 4.3	790	80.3 ± 5.6	339
Female [%]	54.4	790	23.3	339
Body-mass index [kg/m²]	27.7 ± 4.3	790	28.8 ± 4.9	335
Diabetes [%]	17.3	790	31.3	339
Hypertension [%]	72.1	788	76.6	338
Tobacco use (present/past) [%]	41.0	790	53.1	339
Low-density lipoprotein [mg/dl]	148.2 ± 33.8	701	139.1 ± 34.2	285
Coronary artery disease (self-reported) [%]	11.8	790	29.6	338
Heart failure (self-reported) [%]	11.5	788	21.7	336
$eGFR_{crea}$ [ml/min/1.73m²]	70.4 ± 14.04	779	59.5 ± 17.2	337
NT-proBNP [pg/ml]	265.6 ± 355.5	790	963.3 ± 2349.0	339
Heart rate [beats per minute]	69.0 ± 11.0		67.8 ± 11.8	338
Regular rhythm [%]	93.0	599	81.0	248
LVMi [g/m²]	103.6 ± 28.1	472	121.4 ± 36.31	179
Left atrial volume/BSA [ml/m²]	37.5 ± 14.0	575	44.5 ± 18.1	235
E/mean e'	11.1 ± 3.4	530	12.3 ± 4.5	210
Diastolic dysfunction [%]	60.6	563	74.9	227
Ejection fraction [%]	60.7 ± 6.9	582	58.9 ± 9.2	237

Table 4: Characteristics of the study sample divided by the recommended rule-out cut-off of high-sensitivity troponin T for non-ST-segment elevation myocardial infarction in case of no relevant increase within 2 hours (14ng/l)

Shown are mean and standard deviation or proportions.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine. NT-proBNP: N-terminal prohormone of brain natriuretic peptide. LVMi: ratio of left ventricular mass to body surface area. BSA: body surface area. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. Diastolic dysfunction determined according to.

	n	99th hsTnT percentile [95% CI]	95th hsTnT percentile [95% CI]	Proportion below hsTnT 14ng/l
All	1,129	54 [44 - 74]	29 [26 – 31]	68
Stratified by sex				
Women	509	38 [27 - 79]	22 [20 - 23]	82
Men	620	64 [46 - 102]	31 [29 - 36]	57
Stratified by sex and age				
Women 70-79 yrs	375	29 [23 - 58]	19 [15 - 21]	88
Women 80-95 yrs	134	67 [39 - 79]	27 [22 - 39]	66
Men 70-79 yrs	433	70 [42 - 281]	30 [26 - 33]	67
Men 80-95 yrs	187	59 [52 - 75]	37 [31 - 46]	34
Stratified by kidney function				
eGFR ≥ 60	778	33 [30 - 36]	24 [22 - 26]	76
eGFR < 60	338	77 [56 - 308]	44 [34 - 53]	50
Subcohort I				
All	618	32 [28 - 33]	22 [21 - 25]	79
Stratified by sex				
Women	289	25 [21 - 41]	17 [15 - 20]	90
Men	329	32 [30 - 33]	25 [23 - 28]	70
Stratified by age group				
70-79 yrs	507	30 [26 - 33]	21 [19 - 23]	83
80-95 yrs	111	40 [31 - 41]	28 [23 - 32]	62
Subcohort II				
All	408	31 [26 - 33]	22 [17 - 25]	83
Stratified by sex				
Women	191	22 [21 - 22]	16 [14 - 19]	92
Men	217	33 [30 - 33]	25 [22 - 28]	76
Stratified by age group				
70-79 yrs	335	29 [24 - 32]	19 [15 - 21]	88
80-95 yrs	73	N/A	27 [22 - 32]	62
Subcohort III				
All	106	29 [20 - 29]	17 [14 - 24]	91
Stratified by sex				
Women	52	N/A	17 [11 - 19]	94
Men	54	N/A	20 [15 - 29]	87
Stratified by age group				
70-79 yrs	95	N/A	14 [12 - 20]	95
80-95 yrs	11	N/A	N/A	55

Table 5: The 99th and 95th percentiles of high-sensitivity troponin T and percentiles corresponding to the recommended rule-out cut-off for non-ST-segment elevation myocardial infarction (14ng/l).

Shown are 99th and 95th percentiles with 95% confidence intervals in the entire AugUR study sample **(all)** with further stratification for sex, age and renal function, as well as in subcohorts free of overt heart disease and impaired renal function **(subcohort 1)**, comorbidities associated with elevated hsTroponinT (diabetes, obesity; **subcohort 2**) and subtle cardiovascular disease measurable by echocardiography **(subcohort 3)**.

Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2).

Subcohort II: as subcohort I, additionally free of diabetes and obesity (body-mass index <30 kg/m^2).

Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

Left ventricular hypertrophy: left ventricular mass to body surface area >115g/m2 for men / 95g/m2 for women. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. EF: ejection fraction. $eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Overall, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction was below 99%, ranging from 34% to 88% in different sex-, age-, and eGFR-groups.

Specificity of the rule-out upper reference limit (14ng/l) in healthy subgroups

Next, we evaluated the specificity of 14ng/l hsTnT cut-off value in a healthy subgroup of our study participants **(Table 5)**: in subjects free of clinical coronary artery disease, heart failure or impaired renal function (subcohort I, n=618) specificity increased to 79% compared to the 68% in all participants. This proportion barely changed by additional exclusion of diabetic or obese participants (83%; subcohort II, n=408). To further account for subtle, asymptomatic cardiac disorders, echocardiographic data was used to finally analyse a subgroup additionally free of any of the following: (i) no left ventricular hypertrophy (left ventricular mass to body surface area >115g/m^2 for men; 95g/m^2 for women)[19], (ii) no elevated left ventricular filling pressure (E/mean e' > 14)[22] and (iii) no left ventricular systolic dysfunction (ejection fraction < 50%)[25]. In the resulting subgroup (subgroup III, n=106), specificity increased to 91%, whilst remaining poor in participants above 79 years of age (55%).

Together, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction ranged between 79% to 91% in the healthy subgroups.

Upper percentiles in the elderly

As results of the low specificity corresponding to 14ng/l hsTnT in our study participants, we were interested, which value of hsTnT reflected the 99th and 95th percentiles in our elderly individuals. The 99th percentile of the entire study sample was 54ng/l, showing higher levels in men and impaired kidney function (**Table 5**). Excluding overt cardiac disease and renal dysfunction (subcohort I), the 99th percentile was considerably lower (32ng/l). Further exclusion of diabetes or obesity (subcohort II) and of subtle, subclinical cardiac disorders

detected by echocardiography (subcohort III) did only slightly lower the 99th percentile (31 ng/l or 29 ng/l, respectively).

Since age, sex and kidney function defined relevant strata for hsTnT levels throughout our analyses and are usually known parameters in the setting of hospital admission for suspected myocardial infarction, we provide our 95th percentile values in the corresponding subcohorts and separately by these strata **(Table 6)**.

hsTroponinT [ng/l]	**Women**				**Men**			
Age	**70 - 79**		**80 - 95**		**70 - 79**		**80 - 95**	
	95th percentile	n	95th percentile	n	95th percentile	n	95th percentile	n
eGFR ≥ 60	17.4	293	22.6	66	24.4	327	29.2	92
eGFR < 60	21.6	75	35.1	66	57.0	103	47.7	94

Table 6: Upper limit (95th percentile) of blood ranges for high-sensitivity troponin T in the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Discussion

In our study sample comprising 1,129 mobile, elderly participants free from symptoms of acute myocardial infarction, hsTnT-levels increase with age, are considerably higher in men than in women and rise in participants with impaired renal function. The specificity of the endorsed rule-out upper reference limit of hsTnT (14ng/l) is just 70% in the entire study sample, while the cut-off from guidelines was set to reflect 99% specificity[1,4,5]. A particularly low specificity, at 34%, is found among men aged 80 years or older. Correspondingly, all 99th percentiles in our entire study sample as well as in healthy subcohorts are substantially above the cut-off of 14ng/l. Finally, we provide hsTnT-values reflecting a specificity of 95% in our study stratified for sex, age and kidney function to supply physicians with an estimate of specificity in their ageing patients.

Distribution of hsTnT in the elderly

hsTnT-assay was established in healthy study samples a decade ago [4,5]. The 99th percentile of the hsTnT-distribution gained soon major interest, as it turned out to be a sufficient upper reference limit for rule-out of acute myocardial infarction in numerous further analyses [1,6,7]. One of the first studies assessing the hsTnT-assay reported an estimated 99th percentile of 13.5ng/l in a pooled reference population of 616 subjects with mean age of 44 years and age ranging from 20 to 71 years [4]. A second study sample comprised 533 participants with a mean age of 37 years including 1 subject older than 70 years and reported an 99th percentile of 14.2ng/l [5]. However, a joint analyses of data from large, population-based studies including the Dallas Heart Study (DHS), the Atherosclerosis Risk in Communities Study (ARIC) and the Cardiovascular Health Study challenged uniform cut-off values, as the authors reported considerable sex- and age-differences for 99th percentile values[8]. Accordingly, in the Generation Scotland Scottish Family Health Study (GS:SFHS) entailing 19,501 individuals, the 14ng/l-value showed a good fit in age groups below 60 years, whereas the 99th percentile is about 3-fold higher in participants above 60 years of age [9,10]. The increasing hsTnT levels in the age groups beyond 60 years are of particular clinical interest, as they correspond to the median age of patients suffering from troponin positive myocardial

infarction in emergency departments, e.g. 70 years (IQR 19.9 years) in the German chest pain unit registry [11]. Nevertheless, the published data on hsTnT-distribution in the elderly is scarce and hitherto derived from population-based studies, in which recruitment of younger participants prevailed by far as in DHS, ARIC and GS:SFHS [8–10]. Thus, our study complements the discussed published data by focusing on the very old (76.7 years, IQR 7.2 years, age ranging from 70 to 95years) and provides relevant evidence for estimating the hsTnT distribution in the elderly: the recommended rule-out upper reference limit of hsTnT (14ng/l) is just the 70th percentile in our entire study sample of 1,129 individuals and is particularly low, at the 34th percentile, among men aged 80 years or older. The 99th percentile in our entire study sample is four-fold higher than 14ng/l.

Indeed, these values have to be interpreted with caution, as several illnesses with increasing age-dependent prevalence are *per se* associated with elevated hsTnT-levels, e.g. impaired kidney function, obesity, diabetes mellitus type II and irregular heart rhythm [7,9,26]. Furthermore, elevated hsTnT-levels are linked to signs of subtle, non-overt cardiac disease with increasing prevalence in the elderly, as increased left ventricular filling pressure [27] and left ventricular hypertrophy[28]. However, even in our reasonably healthy sub-cohort free of pre-existing cardiac disease, i.e., free of all discussed comorbidities associated with higher hsTnT-levels as well as of echocardiographic signs of non-overt heart disease, the 99th percentile is calculated as 29ng/l and thus twice as high as the recommended rule-out cut-off value of 14ng/l, which just represents the 91st percentile even in the very healthy elderly.

Clinical implications

In chest pain patients, elevated age and comorbidities are highly prevalent, as depicted by the German chest pain unit registry[29]. Both are associated with increased risk of coronary artery disease and entail a raising incidence of non-ST-segment elevation myocardial infarction[6,30]. As missed acute cardiac ischemia is associated with considerable mortality [31], the sensitivity for hsTnT-rule out cut-off is intended to be high. Thus, whereas low sensitivity of the hsTnT-rule-out cut-off value implies elevated mortality, ramifications of low specificity are less obvious: even in the absence of acute myocardial infarction, age and comorbidities as well as elevated hsTnT-values are frequent in chest pain unit patients [29]:

retrospective analyses of 3,219 emergency patients reported 41.5% of subjects aged older than 69 years without acute coronary syndrome above the upper reference limit of 14ng/l[12]. This is in line with retrospective data from the emergency department of the University Hospital Lund, Sweden, where the specificity of the cut-off of 14ng/l in chest pain patients aged 75 years or older was reported with 38%[13]. In patients with clinical suspicion of myocardial infarction and hsTnT-value above 14ng/l, current guidelines recommend a second hsTnT-determination after two hours to look for hsTnT-dynamics. Even if hsTnT-values do not further increase, an observational time of at least four hours in the emergency department entailing a third hsTnT-determination after 3 hours and an echocardiography is endorsed[1] before transfer to a cardiologic ward. Invasive coronary angiography is considered in case of high degree of clinical suspicion of myocardial infarction, while in patients with low-to-intermediate likelihood further non-invasive imaging is recommended by the ESC guidelines[1]. A recent collaborative analysis of three large diagnostic studies used the ESC algorithm and highlighted the consequences of decreasing specificity in higher age: 3,123 patients admitted for suspicion of acute myocardial infarction were prospectively enrolled. The percentage of patients aged 70 years or older remaining in the observe zone and requiring additional diagnostic testing was almost twice as high as in middle-aged (≥55 to < 70 years) and more than four times as high as in patients younger than 55 years[6]. Together, low specificity of the baseline rule-out value implies longer observational time in the emergency department, hospitalisation and additional examinations for patients. Particularly the hazard of in the end unnecessary invasive coronary angiography is to consider owing to high risk of periprocedural events in elderly and multimorbid individuals [32]. Concerning the health system, long observation times and unnecessary diagnostics impair the workflow and resource management in emergency departments, which is recently more appreciated due to the current pandemic of coronavirus disease 2019 (COVID-19).

However, age-specific higher rule-out cut-off values did barely improve the diagnostic performance of the ESC algorithm, but increased diagnostic complexity [6]. Therefore, the 2020 ESC guidelines continue to recommend uniform cut-off concentrations, whilst stressing the importance of an integrative decision pathway based on full clinical assessment, electrocardiogram, hsTroponin-levels and non-invasive imaging[1]. To advance interpretation of the jigsaw piece “hsTnT” in clinical decision making, our study provides specificity data of the uniform rule-out cut-off value of 14ng/l as well as age-specific 99th percentiles of hsTnT

for different strata (age, sex, renal function, history of cardiac disease, regular left ventricular shape and function) in the mobile elderly population aged 70 years or older. The 2020 ESC guidelines limit the recommendation of uniform cut-off-concentrations, until further population-based and clinical data and information technology tools allow to calculate individual reference values based on age and comorbidities. We may report data from the first population-based study, which exclusively focusses on elderly individuals and comprises measurement of hsTnT as well as echocardiography. Our results may contribute to the necessary database comprising epidemiologic data for further meta-analyses and computation of individual risk. For this purpose, we provide extensive data on hsTnT distribution overall and in a variety of strata for this focus group that is the most prevalent in emergency decision making.

Limitations:

We analysed the specificity of hsTnT under the assumption, that none of the AugUR participants had acute myocardial infarction by design. The current guideline definition of acute myocardial infarction entails cardiomyocyte necrosis in a clinical setting consistent with acute myocardial ischaemia[1]. The setting of our study did not at all correspond to acute myocardial infarction: the voluntary, mobile, elderly participants travelled on their own to the study side and were mentally as well as physically fit to go through the approximately three hours of study program without substantial exhaustion. None reported on specific symptoms during the study visit. It is naturally in the nature of myocardial ischaemia, that a study participant could have nevertheless suffered from silent infarction during the study visit. However, given the fact that 30% of participants had hsTnT-values above 14ng/l, a relevant bias of our data due to the rare event of acute, silent infarction during the study visit is not plausible.

Concerning the echocardiographic measurements, our study lacks three-dimensional data acquisition. Consequently, left ventricular mass was determined by the linear method using two-dimensional guided M-Mode in the parasternal long axis view, which relies on assumptions of standardised left ventricular geometry and might be inaccurate in abnormally shaped ventricles and localised hypertrophy. However, the current guidelines of the European Association of Cardiovascular Imaging still explicitly recommend the linear method for large population studies[19,33].

Conclusion

In the elderly population aged at least 70 years, hsTnT-levels continue to raise with age, whilst sex and renal dysfunction are further relevant strata for hsTnT-concentrations in the elderly. The specificity of the 14ng/l cut-off hsTnT-value is substantially lower than 99%, even in healthy subjects. Our study data emphasize the need of further data and discussions on age-dependent cut-off values and also, within high age-groups, cut-off levels that reflect sex and kidney function.

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Conflict of interest statement:

Roche Diagnostics has provided kits for assessment of hsTnT and NT-proBNP free of charge, but it did not play a role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

Author Contributions:

The following authors made substantial contributions to the conceptualisation or design:

investigation: AD, IMH, KJS, MEZ, CB

methodology: AD, IMH, AL, LSM, SW, RB, KJS, MEZ, CB

Data curation: AD, CB, SW, RB, IMH, KJS, MEZ

Formal analysis: AD, IMH, KJS, MEZ

Interpretation: AD, IMH, AL, LSM, KJS, MEZ

Funding acquisition: IMH, CB, AL, KJS

Supervision: IMH, AL, LSM, KJS

Validation: AD, CB, IMH, AL, LSM, KJS, MEZ

Writing (original draft preparation): AD

All authors contributed to the reviewing and editing of the manuscript.

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FIGURES

Figure 1. Values of high-sensitivity troponin T in 1,129 participants of the AugUR study by age groups and sex

A box represents the lower (25%) and upper (75%) quartiles with median as a horizontal line within the box. Y-axis shows values on a log10-based scale. hsTnT: high-sensitivity troponin T.

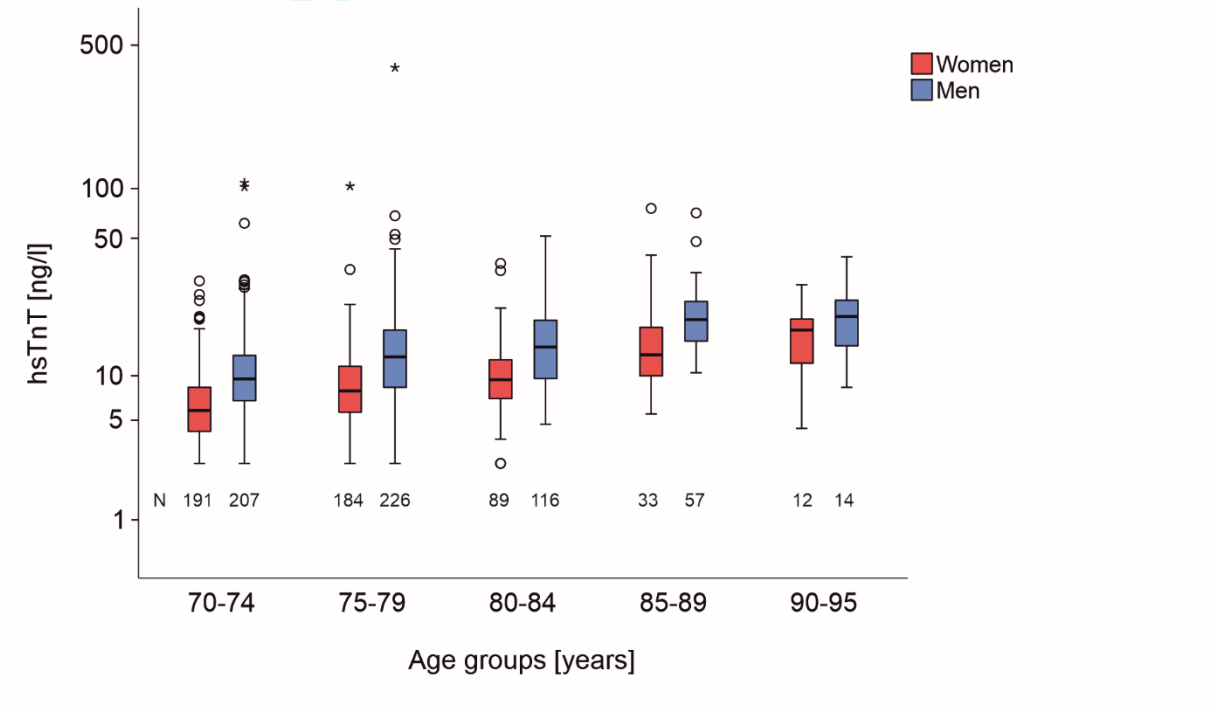


Figure 2. Proportion below and above a high-sensitivity troponin T rule-out cut-off value of 14 ng/l in different AugUR subgroups.

The proportion of negatives according to the rule-out cut-off value of 14ng/l, who are correctly identified as not having acute myocardial infarction, decreases with sex, age and renal function (blue boxes), whilst the rate of false positives increases (orange boxes). Grey boxes represent the commonly accepted false positive rate of 1%.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. hsTnT: high-sensitivity troponin T. Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2). Subcohort II: as subcohort I, additionally free of diabetes and obesity (body-mass index <30 kg/m^2). Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

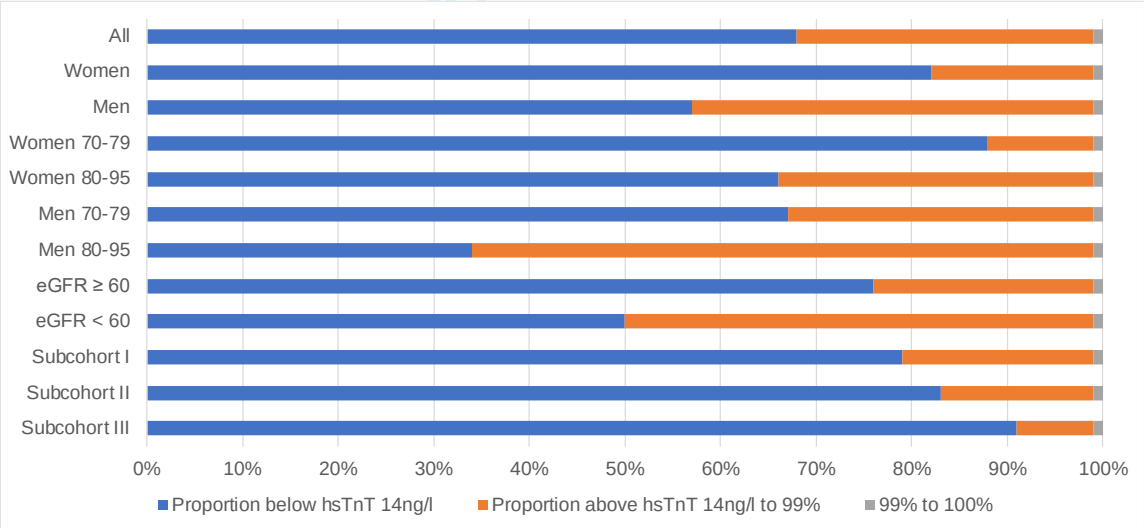
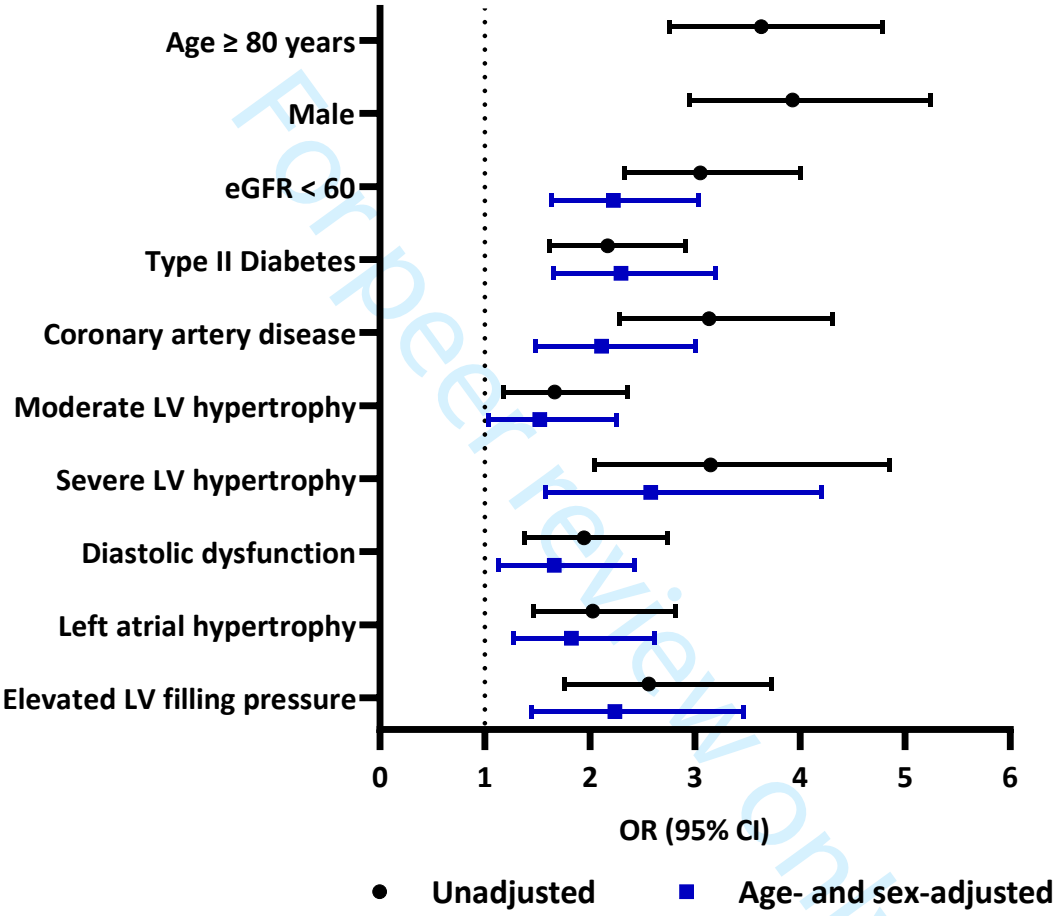


Figure 3. Determinants of elevated high-sensitivity troponin T (>14ng/l)

Odds ratio estimates for high-sensitivity troponinT > 14 ng/l. Simple logistic regression without adjustment and after adjustment for age and sex. Presented are the OR and 95% CI. Dashed line indicates OR=1.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. LV: left ventricular. Elevated LV filling pressure: E/e' >14.



Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cross sectionalreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5

Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5
	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	22
Study size	#10	Explain how the study size was arrived at	5 and 8
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7 and 14-15
Statistical methods	#12c	Explain how missing data were addressed	n/a
Statistical methods	#12d	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	#12e	Describe any sensitivity analyses	7 and 14-15
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
Participants	#13b	Give reasons for non-participation at each stage	5 and 8

Participants	#13c	Consider use of a flow diagram	n/a
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	8 and 12
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 3 + caption
Main results	#16b	Report category boundaries when continuous variables were categorized	10,11,13,17, Fig3+caption
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9-17
Discussion			
Key results	#18	Summarise key results with reference to study objectives	18
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	22
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18-22
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18-22
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

Notes:

- 12b: 7 and 14-15
- 12e: 7 and 14-15
- 16a: Figure 3 + caption
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Distribution and specificity of high-sensitivity cardiac troponin T in old aged without acute cardiac conditions: cross-sectional results from the population-based AugUR study

Alexander Dietl[1], Martina E. Zimmermann[2], Caroline Brandl[2,3], Stefan Wallner[4], Ralph Burkhardt[4], Lars S. Maier[1], Andreas Luchner[5], Iris M. Heid[2], Klaus J. Stark[2]

[1] Department of Internal Medicine II, University Hospital Regensburg, Regensburg, Germany

[2] Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany

[3] Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany

[4] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

[5] Department of Cardiology, Krankenhaus der Barmherzigen Brueder, Regensburg, Germany

Corresponding author:

Dr. Alexander Dietl

University Hospital Regensburg

Department of Internal Medicine II

D-93042 Regensburg

Germany

Email : alexander.dietl@ukr.de

Phone : +49 941 944 0

Fax : +49 941 944 5212

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Abstract

Objective: European guidelines recommended a uniform upper-reference limit of high sensitivity cardiac troponinT (hsTnT) to rule-out non-ST-segment-elevation myocardial infarction. Our study aimed to provide a hsTnT reference-distribution and to assess the specificity of the 14ng/l cut-off value in the mobile population ≥70years of age.

Design: A cross-sectional analysis was performed in the German AugUR study (**A**ltersbezogene **U**ntersuchungen zur **G**esundheit der **U**niversity of **R**egensburg).

Setting: Study population was the mobile population aged 70+ years living in the city and county of Regensburg, Germany.

Participants: A random sample was derived from the local population registries of residence. Of the 5,644 individuals invited, 1,133 participated (response ratio=20.1%). All participants came to the study centre and were mentally and physically mobile to conduct the protocol (face-to-face interview, blood draw, standardized transthoracic echocardiography). None of the participants was in an acute state of myocardial infarction.

Results: Among the 1,129 individuals with hsTnT measurements (overall median=10.0ng/l, interquartile range [IQR]=8.0), hsTnT was higher among the older individuals and higher among men (men 70-74years median=9.6ng/l, IQR=5.9ng/l; men 90-95years median=21.2ng/l IQR=11.4ng/l; women 70-74 years median=6.3ng/l, IQR=4.0ng/l; women 90-95years median=18.0ng/l, IQR=10.0ng/l). In participants with impaired kidney function ($eGFR_{crea}$<60ml/min/1.73m^2), hsTnT was elevated (median=13.6ng/l, IQR=11.2ng/l). Specificity of recommended upper-reference limit, 14ng/l, is 68%. Most false positives were among men aged >79years (specificity=34%). In a healthy subgroup (n=96, none of the following: overt heart disease, impaired renal function, blood pressure > 160/100mmHg, left ventricular hypertrophy, diastolic/systolic dysfunction), specificity was 90%.

Conclusion: In the elderly population without acute myocardial infarction, hsTnT further increases with age showing different levels for men and women. The specificity of the 14ng/l cut-off is considerably lower than 99%, even in healthy subjects.

Article Summary

Strengths and limitations of this study

Population-based approach: A major strength of our study is the population-based approach focussed on the age group, which is most often seen in chest pain units.

Appropriate design: The study was a-priori designed to determine reference values of biomarker incorporating thorough protocols for collection of serum and elaborated biobanking.

Rigorous conduct: The study protocol entailed firmly standardised procedures as well as the conduct by trained, experienced and quality-controlled staff.

Cardiac imaging: Echocardiography was performed according to current European and American guidelines following in advance defined standard operating procedures.

Focused on just one ethnic group: As the recruitment area in South-Eastern Germany implies a largely Caucasian population, we cannot report on high-sensitivity troponinT concentrations in further ethnicities.

Introduction

High sensitivity cardiac troponin T (hsTnT) is a sensitive marker of cardiomyocyte injury indicating myocardial damage resulting from, e.g. myocardial ischaemia, pulmonary embolism, myocarditis or Takotsubo syndrome[1–3]. In chest pain patients, hsTnT constitutes a mainstay for diagnosis of non-ST-segment elevation myocardial infarction. The 2020 Guidelines of the European Society of Cardiology for the management of acute coronary syndromes continue to recommend a uniform cut-off concentration of 14ng/l for rule-out of non-ST-elevation acute myocardial infarction in the 0/2-hour protocol. This hsTnT-value was initially derived from a pooled reference population of 616 subjects (mean age 44 years) and a study sample comprising 533 individuals (mean age 37 years), in which a value of 14ng/l signified approximately the 99th percentile of hsTnT-distribution [4,5]. In several further analyses, it turned out to be a sufficiently sensitive upper reference limit for rule-out of acute myocardial infarction in the emergency department [1,6,7].

While high sensitivity is crucial for a biomarker diagnosing an acute, life-threatening disease with immediate options for effective intervention, specificity can also be important: low specificity implies a large proportion of unnecessary examinations, hospitalization, and cardiac catherization along with risks of serious complications[6]. Older and multimorbid patients carry a particularly elevated risk for complications from percutaneous coronary intervention[8], which emphasizes the relevance of specificity particularly for the old aged. To this extent, large population-based studies have challenged uniform cut-off values due to considerable sex- and age-differences in hsTnT-distribution with decreasing specificity by age [9–11]. The dependency of hsTnT-concentrations on age implies major clinical impact: most chest pain patients are at advanced age [12] and the decreasing specificity of the uniform cut-off by age yields a growing number of false-positive results in the elderly [13,14]. Despite being the primary clinical target population for the application of these cut-off values, the elderly are less captured in published data on hsTnT-distribution [9–11]. This gap can be attributed to the specific needs of the elderly, which often hamper their participation in population-based studies or prompt general studies to exclude individuals above the age of, e.g., 70 years[15,16]. The aims of our analyses were to understand the distribution for hsTnT-values in the mobile population ≥70 years of age without acute cardiac disease and to quantify the specificity of the 14ng/l cut-off value at old age. We report on our cross-sectional data from

1,129 participants of the German AugUR study (**A**ltersbezogene **U**ntersuchungen zur **G**esundheit der **U**niversity of **R**egensburg), which focused on the mobile population ≥70years of age. The study protocol entailed a face-to-face interview, collection of serum samples and a standardized transthoracic echocardiography enabling a thorough assessment of even subtle subclinical cardiac disorders.

Methods

Study sample

The design of the German AugUR study has been described in detail previously[17]. Briefly, we recruited inhabitants at least 70 years of age in the city of Regensburg, Germany, and selected nearby counties. The local registries of residence provided a random sample of 5,971 subjects' postal addresses, who were invited by mail. Of these, (i) 327 persons were not contactable, as they had moved outside the study region or had meanwhile died, (ii) 3,187 persons did not respond, (iii) 1,324 responded negatively (i.e., declined participation by phone or in writing), and (iv) 1,133 participated (response among the 5,644 contactable =20.1%). For 402 non-participants, the specified reasons for denial were: 56.5% too ill, 6.2% no time, 20.1% no interest, 17.2% other. The 1,133 participants were able to come to the study centre at the University Medical Centre, to walk around independently, to answer all interview questions personally, and to conduct a two-hour study program including non-invasive medical exams. Thus, all participants had no acute cardiac events, were physically mobile and mentally fit. We consider our participants to reflect the "mobile" old aged population.

Patient and Public Involvement Statement

The AugUR survey is an epidemiologic, cross-sectional study, inviting a random sample of the general population aged 70 or more years. Accordingly, no specific group of patients is involved. Results are published in scientific journals and presented on the web page of the AugUR study (https://www.uni-regensburg.de/medizin/epidemiologie-praeventivmedizin/genetische-epidemiologie/augur/index.html). Specific results are accessible for every participant upon reasonable request.

Ethics statement

The study protocol, study procedures, and data protection strategy were all approved by the Ethics Committee of the University of Regensburg, Germany (vote 12-101-0258). All study

participants provided written consent after being informed about the study. The study was conducted according to the principles expressed in the Declaration of Helsinki. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

General data assessment

Sociodemographic factors, smoking behaviour, medication use and cardiovascular medical history (including existence, history, and time onset of cardiovascular diseases and interventions) were assessed in a standardised face-to-face interview by trained staff. Blood pressure was measured using an automatic device (Omron M10-IT; Omron Healthcare, Kyoto, Japan), pulse rate was determined by palpation after five minutes of resting time. Blood pressure was measured three times and the average of the second and third measurement were computed for further analyses.

Assessment of cardiac morphology and function by echocardiography

In order to assess even subclinical cardiac disorders, transthoracic echocardiography was performed using a commercially available ultrasound unit (HP Sonos 5500 with 2-4 MHz probe; Philips, Eindhoven, The Netherlands). The stored tracings were evaluated post hoc using analytical software Xcelera R3.2L1 Version 3.2.1.520 – 2011 (Philips Medical Systems, Amsterdam, Netherlands) as previously described[18]. The echocardiographic program focused on left atrial and ventricular morphology and function accounting for chamber-specific cardiac remodelling processes[19] according to the current guidelines[20]: left atrial volume was determined by two-dimensional volumetric measurement based on tracings of the blood-tissue interface in apical four-chamber view. M-Mode measurements for calculating left ventricular mass were obtained from parasternal long axis view and determined perpendicular to the left ventricular axis. Left ventricular mass was computed by the Devereux formula[21]. Left atrial volume and left ventricular mass were indexed to body-surface area approximated by DuBois' formula[22]. To estimate left ventricular filling pressures, the ratio

of the transmitral early peak velocity by pulsed wave Doppler (E) over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler (mean e') was determined (E/mean e'). Left ventricular diastolic dysfunction was evaluated according to recent recommendations[23]. Systolic function was assessed as ejection fraction estimated by the modified Simpson's method[20] based on monoplanar measurements in the apical four chamber view. Each of the measurements used for further analyses was repeated three times for regular rhythm and ten times in case of arrhythmia to reduce random error.

High-sensitivity Troponin T and NT-proBNP measurements

Collection and procession of biosamples were conducted following standard operation procedures developed for this study based on established methods and recommendations[24]. Deviations from these standard operation procedures (e.g., extended sample handling at room temperature) were recorded and linked with the biosample information. All samples were processed immediately and kept on dry ice before final storage at the end of the day. Identification, assignment and link to electronic case report form (eCRF) data for biosamples including 2D-barcoded tubes were managed by self-developed integrated software.

Non-fasting blood samples were drawn in a sitting position after at least five minutes of resting. Mild venous stasis was applied for a maximum duration of one minute. Whole blood was taken using a 21G multifly needle. Two samples were used for ad hoc analysis. Serum tubes with clot activator were left in upright position for 30 minutes after blood draw and were centrifuged at 2,000 g for 15 minutes at room temperature to separate serum from the cellular fraction as soon as possible. Supernatants from serum tubes were transferred to 2D-barcoded tubes for storage at -80°C.

Measurements for hsTnT and N-terminal prohormone B-type (brain) natriuretic peptide (NT-proBNP) were conducted in stored serum samples by the Department of Clinical Chemistry and Laboratory Medicine of the University Hospital Regensburg on a cobas e411 (Roche Diagnostics, Rotkreuz, Switzerland). After measurement, data were exported from SWISSLAB (NEXUS SWISSLAB GmbH, Berlin, Germany) in Excel format and processed with Microsoft Access 2019 (Microsoft Corporation, Redmond, WA, USA), SAS 9.4 (SAS Institute Inc., Cary,

NC, USA) and SPSS 25.0.0.2 (IBM Corporation, Armonk, NY, USA.). 31 values for hsTnT [ng/l or pg/ml] were on the lower detection limit of "<3" ng/l. Those results were winsorised to "2.9" to discriminate from true "3.0". For NT-proBNP [pg/ml], no values with extremes beyond specified measurement range (5-35,000 pg/ml) were detected.

Statistical methods

Continuous variables are reported as mean and standard deviation (SD) or as median with interquartile range (IQR) and different percentiles. Estimates of confidence intervals for 99th and 95th percentiles were derived by bootstrap analysis using bias corrected and accelerated intervals. Categorical variables are reported as proportions. Odds ratio estimates for hsTnT-values > versus <= 14ng/l were computed by simple logistic regression for each of the covariates separately: age, male sex, impaired kidney function, type II diabetes, history of coronary artery disease, left ventricular hypertrophy, diastolic dysfunction, left atrial hypertrophy and elevated filling pressure (defined as E/e'>14). This was repeated adjusting for age and sex, as applicable. We used the STROBE cross sectional checklist when writing our report[25]. All analyses were carried out with SPSS 25.0.0.2 (IBM, Armonk, USA).

Results

Characteristics of the study sample

1,129 participants out of 1,133 showed valid hsTnT-values and were included for further analyses. Age ranged from 70.3 to 95.0 years, with a median of 76.7 years (interquartile range [IQR] 7.2 years). Demographic, clinical and laboratory characteristics are shown in **Table 1**. Of note, all individuals came walking to the study centre at the University medical centre, participated in the two-hour study program with little exhaustion mentally or physically and can thus be considered mobile elderly. None of the participants had any sign of acute cardiac condition, particularly myocardial infarction, throughout the study visit. While our participants were all relatively healthy by design, they included medical conditions to the extent as one expects from the mobile population of that age.

Characteristics	Women (n=509)	Men (n=620)
Age [years]	77.34 ± 5.02	77.88 ± 5.06
Body-mass index [kg/m^2]	27.8 ± 5.0	28.2 ± 4.0
Diabetes [%]	19.4	23.2
Hypertension [%]	74.2	72.9
Coronary artery disease [%]	9.8	23.1
Heart failure [%]	16.0	13.5
Tobacco use (present/past) [%]	25.5	60.3
$eGFR_{crea}$ [ml/min/1.73m^2]	68.5 ± 16.2	66.1 ± 16.4

Table 1: Baseline characteristics of the study sample

Shown are mean and standard deviation or proportions for the 1,129 subjects separately for women and men.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Distribution of hsTnT-values by age, sex and glomerular filtration rate

First, we looked at the distribution of hsTnT levels by age groups and sex **(Figure 1)**. HsTnT-values increased with age and were higher in men than in women (**Table 2**). Further, we report on values separately for normal and reduced glomerular filtration rate (eGFR≥ vs. < 60ml/min per 1.73m^2, derived from serum creatinine, **Table 3**).

For actual diagnosis of acute non-ST-elevation myocardial infarction in symptomatic patients, the 2020 Guidelines of the European Society of Cardiology endorse a rule-in hsTnT-cut-off concentration of 52ng/l, which implies immediate referral of chest pain patients to invasive diagnostics[1]. In 13 subjects (1,2%) of our study, hsTnT was measured above this rule-in cut-off (≥ 52ng/l) with a median of 72.1ng/l (IQR 46.9) and a maximum of 421ng/l.

Specificity of the rule-out upper reference limit (14ng/l) in the mobile elderly

Next, we intended to estimate the specificity of the endorsed rule-out upper reference limit of hsTnT [1] in our mobile elderly individuals considered free of acute myocardial infarction **(Figure 2).** Applying the recommended cut-off value of 14ng/l, 70% (790/1,129 subjects) of our study participants were below this cut-off. Main determinants of hsTnT-values above 14ng/l were age, male sex, impaired kidney function, type II diabetes, history of coronary artery disease, left ventricular hypertrophy, diastolic dysfunction, left atrial hypertrophy and elevated filling pressure (E/e'>14, **Figure 3**). As this cut-off was defined as the 99th percentile of reference samples without acute myocardial infarction in the attempt to yield 99% specificity [1,4,5], this is in line with the notion that, among our study participants, 70% were correctly identified (true negative for acute myocardial infarction), but 30% (339/1,129) were not (false-positive). These 339 individuals showed a median level of 19.4ng/l (IQR 9.0). They were older, more likely men and more likely with diabetes, stable coronary artery disease, heart failure or impaired kidney function than the 790 individuals below the cut-off (**Table 4**). Regarding echocardiographic measurements, elevated left ventricular mass was detected.

Further stratification revealed a particularly low specificity for the 14ng/l hsTnT level in men (57%) as well as in subjects with impaired kidney function (50% for eGFR < 60 ml/min/1.73m^2) and bottommost in men aged 80 years or older (34%, **Table 5**).

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
Women n	191	184	89	33	12	509
Mean ± SD	7.54 ± 4.56	9.76 ± 8.51	11.03 ± 6.32	16.66 ± 13.62	17.02 ± 6.97	9.77 ± 7.75
Minimum	2.9	2.9	2.9	6.0	4.9	2.9
5th percentile	2.9	2.9	4.5	6.2	4.9	3.1
10th percentile	3.3	3.6	5.2	6.9	6.4	4.0
25th percentile	4.7	6.1	7.2	9.7	11.0	5.7
Median	6.3	8.2	9.5	13.1	18.0	8.0
75th percentile	8.7	11.3	13.0	18.9	2.10	11.5
90th percentile	13.5	15.1	18.7	27.8	28.7	17.4
95th percentile	16.1	19.7	22.5	55.1	-	21.5
Maximum	32.8	102.8	40.6	78.8	31.3	102.8
Men n	207	226	116	57	14	620
Mean ± SD	11.96 ± 11.49	16.31 ± 28.60	16.71 ± 9.87	21.89 ± 10.31	21.16 ± 8.98	15.56 ± 19.49
Minimum	2.9	2.9	5.2	10.4	8.6	2.9
5th percentile	4.8	5.2	6.6	11.3	8.6	5.3
10th percentile	5.6	6.2	7.5	12.5	9.4	6.1
25th percentile	7.2	8.6	9.6	15.5	14.6	8.3
Median	9.6	12.8	14.5	20.4	21.2	12.3
75th percentile	13.1	18.0	20.3	25.6	26.0	18.1
90th percentile	18.4	24.5	30.2	30.2	36.1	26.4
95th percentile	28.4	30.4	37.7	38.0	-	31.3
Maximum	107.1	421.3	56.5	74.6	44.0	421.3

Table 2: hsTroponinT values [ng/l] by age groups and sex in 1,129 participants of the AugUR stud

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
$eGFR_{crea}$ ≥60 n	322	298	113	35	10	778
Mean ± SD	9.16 ± 7.68	10.93 ± 7.79	12.31 ± 6.42	17.31 ± 7.29	14.91 ± 14.75	10.74 ± 7.74
Minimum	2.9	2.9	2.9	6.3	4.9	2.9
5th percentile	3.1	3.1	5.2	6.5	4.9	3.2
10th percentile	4.0	4.7	5.8	8.3	5.3	4.5
25th percentile	5.5	6.9	7.9	11.1	9.8	6.3
Median	7.4	9.2	10.7	16.8	14.8	8.9
75th percentile	10.4	13.2	15.3	22.4	17.8	13.2
90th percentile	14.7	18.8	21.4	28.6	27.5	18.5
95th percentile	21.0	22.6	24.7	29.7	-	23.5
Maximum	107.1	102.8	40.6	33.1	28.2	107.1
$eGFR_{crea}$ <60 n	70	108	90	54	16	338
Mean ± SD	13.07 ± 13.85	20.38 ± 40.60	16.55 ± 10.98	21.95 ± 13.74	21.96 ± 8.18	18.17 ± 25.25
Minimum	2.9	3.4	4.7	7.3	9.8	2.9
5th percentile	4.1	4.8	5.5	9.9	9.8	5.3
10th percentile	5.3	6.9	6.9	11.8	10.0	6.8
25th percentile	7.0	9.7	9.1	13.5	19.4	9.4
Median	10.0	13.5	12.2	19.0	21.2	13.6
75th percentile	13.7	19.7	20.8	23.7	25.8	20.6
90th percentile	20.8	29.0	32.1	33.7	35.1	29.3
95th percentile	30.2	47.4	41.3	58.3	-	43.6
Maximum	101.8	421.3	56.5	78.8	44.0	421.3

Table 3: hsTroponinT values [ng/l] by age groups and $eGFR_{crea}$ categories in 1,129 participants of the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m²; a value of 60 was used to determine between good and limited kidney function

hsTroponinT [ng/l]	<14ng/l	n	≥ 14 ng/l	n
Age [years]	76.5 ± 4.3	790	80.3 ± 5.6	339
Female [%]	54.4	790	23.3	339
Body-mass index [kg/m^2]	27.7 ± 4.3	790	28.8 ± 4.9	335
Diabetes [%]	17.3	790	31.3	339
Hypertension [%]	72.1	788	76.6	338
BP <160/100mmHg [%]	92.0	789	89.7	339
Tobacco use (present/past) [%]	41.0	790	53.1	339
Low-density lipoprotein [mg/dl]	148.2 ± 33.8	701	139.1 ± 34.2	285
Coronary artery disease (self-reported) [%]	11.8	790	29.6	338
Heart failure (self-reported) [%]	11.5	788	21.7	336
$eGFR_{crea}$ [ml/min/1.73m^2]	70.4 ± 14.04	779	59.5 ± 17.2	337
NT-proBNP [pg/ml]	265.6 ± 355.5	790	963.3 ± 2349.0	339
Heart rate [beats per minute]	69.0 ± 11.0		67.8 ± 11.8	338
Regular rhythm [%]	93.0	599	81.0	248
LVMi [g/m^2]	103.6 ± 28.1	472	121.4 ± 36.31	179
Left atrial volume/BSA [ml/m^2]	37.5 ± 14.0	575	44.5 ± 18.1	235
E/mean e'	11.1 ± 3.4	530	12.3 ± 4.5	210
Diastolic dysfunction [%]	60.6	563	74.9	227
Ejection fraction [%]	60.7 ± 6.9	582	58.9 ± 9.2	237

Table 4: Characteristics of the study sample divided by the recommended rule-out cut-off of high-sensitivity troponin T for non-ST-segment elevation myocardial infarction in case of no relevant increase within 2 hours (14ng/l)

Shown are mean and standard deviation or proportions.

BP: blood pressure. $eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine. NT-proBNP: N-terminal prohormone of brain natriuretic peptide. LVMi: ratio of left ventricular mass to body surface area. BSA: body surface area. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. Diastolic dysfunction determined according to[23].

	n	99th hsTnT percentile [95% CI]	95th hsTnT percentile [95% CI]	Proportion below hsTnT 14ng/l
All	1,129	54 [44 - 74]	29 [26 – 31]	68
Stratified by sex				
Women	509	38 [27 - 79]	22 [20 - 23]	82
Men	620	64 [46 - 102]*†	31 [29 - 36]*	57
Stratified by sex and age				
Women 70-79 yrs	375	29 [23 - 58]	19 [15 - 21]	88
Women 80-95 yrs	134	67 [39 - 79]	27 [22 - 39]	66
Men 70-79 yrs	433	70 [42 - 281]*‡	30 [26 - 33]*¶	67
Men 80-95 yrs	187	59 [52 - 75]	37 [31 - 46]	34
Stratified by kidney function				
eGFR ≥ 60	778	33 [30 - 36]	24 [22 - 26]	76
eGFR < 60	338	77 [56 - 308]*§	44 [34 - 53]*Δ	50
Subcohort I				
All	618	32 [28 - 33]	22 [21 - 25]	79
Stratified by sex				
Women	289	25 [21 - 41]	17 [15 - 20]	90
Men	329	32 [30 - 33]	25 [23 - 28]	70
Stratified by age group				
70-79 yrs	507	30 [26 - 33]	21 [19 - 23]	83
80-95 yrs	111	40 [31 - 41]	28 [23 - 32]	62
Subcohort II				
All	366	31 [26 - 33]	20 [17 - 23]	83
Stratified by sex				
Women	173	22 [21 - 22]	16 [14 - 20]	90
Men	193	33 [31 - 33]	25 [18 - 29]	77
Stratified by age group				
70-79 yrs	304	30 [22 - 33]	18 [15 - 21]	88
80-95 yrs	62	N/A	29 [22 - 33]	60
Subcohort III				
All	96	N/A	17 [14 - 25]	90
Stratified by sex				
Women	49	N/A	17 [11 - 20]	94
Men	47	N/A	23 [14 - 29]	85
Stratified by age group				
70-79 yrs	86	N/A	14 [12 - 20]	94
80-95 yrs	10	N/A	N/A	50

Table 5: The 99th and 95th percentiles of high-sensitivity troponin T and percentiles corresponding to the recommended rule-out cut-off for non-ST-segment elevation myocardial infarction (14ng/l).

Shown are 99th and 95th percentiles with 95% confidence intervals in the entire AugUR study sample **(all)** with further stratification for sex, age and renal function, as well as in subcohorts free of overt heart disease and impaired renal function **(subcohort 1)**, comorbidities associated with elevated hsTroponinT (diabetes, obesity; **subcohort 2**) and subtle cardiovascular disease measurable by echocardiography **(subcohort 3)**.

Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2).

Subcohort II: as subcohort I, additionally free of diabetes and obesity (body-mass index <30 kg/m^2) with a blood pressure <160/100mmHg at study visit.

Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

*Leave-one-out analyses revealed an influential observation: one man (age 77years, eGFR 59ml/min/1.73, no coronary artery disease, LVMi 117g/m2, EF 65%) exhibited an extraordinarily elevated hsTnT-level of 421ng/l. Excluding it, percentiles and 95% confidence intervals were lowered to †57 [46 - 75], ‡63 [38 - 101] and §74 [55 - 93] for the 99th percentiles in ng/l [95%CI] and 31 [30 - 33], ¶ 29 [26 – 33] and $^{\Delta}$43 [33 – 49] for the 95th percentiles in ng/l [95%CI].

Left ventricular hypertrophy: left ventricular mass to body surface area >115g/m2 for men / 95g/m2 for women. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. EF: ejection fraction. $eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Overall, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction was below 99%, ranging from 34% to 88% in different sex-, age-, and eGFR-groups.

Specificity of the rule-out upper reference limit (14ng/l) in healthy subgroups

Next, we evaluated the specificity of 14ng/l hsTnT cut-off value in a healthy subgroup of our study participants **(Table 5)**: in subjects free of clinical coronary artery disease, heart failure or impaired renal function (subcohort I, n=618) specificity increased to 79% compared to the 68% in all participants. This proportion barely changed by additional exclusion of diabetic and obese participants as well as subjects with a measured blood pressure >160/100mmHg (83%; subcohort II, n=366). To further account for subtle, asymptomatic cardiac disorders, echocardiographic data was used to finally analyse a subgroup additionally free of any of the following: (i) no left ventricular hypertrophy (left ventricular mass to body surface area >115g/m^2 for men; 95g/m^2 for women)[20], (ii) no elevated left ventricular filling pressure (E/mean e' > 14)[23] and (iii) no left ventricular systolic dysfunction (ejection fraction < 50%)[26]. In the resulting subgroup (subgroup III, n=96), specificity increased to 90%, whilst remaining poor in participants above 79 years of age (50%).

Together, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction ranged between 79% to 90% in the healthy subgroups.

Upper percentiles in the elderly

As results of the low specificity corresponding to 14ng/l hsTnT in our study participants, we were interested, which value of hsTnT reflected the 99th and 95th percentiles in our elderly individuals. The 99th percentile of the entire study sample was 54ng/l, showing higher levels in men and impaired kidney function (**Table 5**). Excluding overt cardiac disease and renal dysfunction (subcohort I), the 99th percentile was considerably lower (32ng/l). Further

exclusion of diabetes, obesity and elevated blood pressure (>160/100mmHg, subcohort II) did only slightly lower the 99th percentile (31 ng/l).

Since age, sex and kidney function defined relevant strata for hsTnT levels throughout our analyses and are usually known parameters in the setting of hospital admission for suspected myocardial infarction, we provide our 95th percentile values in the corresponding subcohorts and separately by these strata **(Table 6)**.

hsTroponinT [ng/l]	Women				Men			
Age	**70 - 79**		**80 - 95**		**70 - 79**		**80 - 95**	
	95th percentile	n	95th percentile	n	95th percentile	n	95th percentile	n
eGFR ≥ 60	17.4	293	22.6	66	24.4	327	29.2	92
eGFR < 60	21.6	75	35.1	66	57.0	103	47.7	94

Table 6: Upper limit (95th percentile) of blood ranges for high-sensitivity troponin T in the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Discussion

In our study sample comprising 1,129 mobile, elderly participants free from symptoms of acute myocardial infarction, hsTnT-levels increase with age, are considerably higher in men than in women and rise in participants with impaired renal function. The specificity of the endorsed rule-out upper reference limit of hsTnT (14ng/l) is just 70% in the entire study sample, while the cut-off from guidelines was set to reflect 99% specificity[1,4,5]. A particularly low specificity, at 34%, is found among men aged 80 years or older. Correspondingly, all 99th percentiles in our entire study sample as well as in healthy subcohorts are substantially above the cut-off of 14ng/l. Finally, we provide hsTnT-values reflecting a specificity of 95% in our study stratified for sex, age and kidney function to supply physicians with an estimate of specificity in their ageing patients.

Distribution of hsTnT in the elderly

hsTnT-assay was established in healthy study samples a decade ago [4,5]. The 99th percentile of the hsTnT-distribution gained soon major interest, as it turned out to be a sufficient upper reference limit for rule-out of acute myocardial infarction in numerous further analyses [1,6,7]. One of the first studies assessing the hsTnT-assay reported an estimated 99th percentile of 13.5ng/l in a pooled reference population of 616 subjects with mean age of 44 years and age ranging from 20 to 71 years [4]. A second study sample comprised 533 participants with a mean age of 37 years including 1 subject older than 70 years and reported a 99th percentile of 14.2ng/l [5]. However, a joint analyses of data from large, population-based studies including the Dallas Heart Study (DHS), the Atherosclerosis Risk in Communities Study (ARIC) and the Cardiovascular Health Study challenged uniform cut-off values, as the authors reported considerable sex- and age-differences for 99th percentile values[9]. Accordingly, in the Generation Scotland Scottish Family Health Study (GS:SFHS) entailing 19,501 individuals, the 14ng/l-value showed a good fit in age groups below 60 years, whereas the 99th percentile is about 3-fold higher in participants above 60 years of age [10,11]. The increasing hsTnT levels in the age groups beyond 60 years are of particular clinical interest, as they correspond to the median age of patients suffering from troponin positive myocardial infarction in emergency departments, e.g. 70 years (IQR 19.9 years) in the German chest pain

unit registry [12]. Nevertheless, the published data on hsTnT-distribution in the elderly is scarce and hitherto derived from population-based studies, in which recruitment of younger participants prevailed by far as in DHS, ARIC and GS:SFHS [9–11]. Thus, our study complements the discussed published data by focusing on the very old (76.7 years, IQR 7.2 years, age ranging from 70 to 95years) and provides relevant evidence for estimating the hsTnT distribution in the elderly: the recommended rule-out upper reference limit of hsTnT (14ng/l) is just the 70th percentile in our entire study sample of 1,129 individuals and is particularly low, at the 34th percentile, among men aged 80 years or older. The 99th percentile in our entire study sample is four-fold higher than 14ng/l.

Indeed, these values have to be interpreted with caution, as several illnesses with increasing age-dependent prevalence are *per se* associated with elevated hsTnT-levels, e.g. impaired kidney function, obesity, diabetes mellitus type II and irregular heart rhythm [7,10,27]. Furthermore, elevated hsTnT-levels are linked to elevated blood pressure[28,29] as well as signs of subtle, non-overt cardiac disease with increasing prevalence in the elderly, as increased left ventricular filling pressure [30] and left ventricular hypertrophy[31]. However, even in our reasonably healthy sub-cohort free of pre-existing cardiac disease, i.e., free of all discussed comorbidities and having blood pressure below 160/100mmHg, the 99th percentile is calculated as 31ng/l and thus more than twice as high as the recommended rule-out cut-off value of 14ng/l. In the very healthy sub-cohort, that is additionally free of echocardiographic signs of non-overt heart disease, specificity of the 14ng/l cut-off value is down to 90%.

The effect of age and sex on cut-off specificity is not only clear for hsTnT: Welsh and colleagues[10] compared cardiac troponin T and I in a large general population cohort. Despite the fact, that cardiac troponin T and I are only weakly correlated with each other and show different extent of association with cardiovascular risk factors, the 99th percentiles differ between men and women beyond the age of 70 years for both biomarkers[10].

Clinical implications

In chest pain patients, elevated age and comorbidities are highly prevalent, as depicted by the German chest pain unit registry[32]. Both are associated with increased risk of coronary artery disease and entail a raising incidence of non-ST-segment elevation myocardial

infarction[6,33]. High sensitivity is evidently crucial for a biomarker diagnosing an acute, life-threatening disease: missed acute cardiac ischemia is associated with considerable mortality [34]. Thus, whereas low sensitivity of the hsTnT-rule-out cut-off value implies elevated mortality, ramifications of low specificity are less obvious: even in the absence of acute myocardial infarction, age and comorbidities as well as elevated hsTnT-values are frequent in chest pain unit patients [32]: retrospective analyses of 3,219 emergency patients reported 41.5% of subjects aged older than 69 years without acute coronary syndrome above the upper reference limit of 14ng/l[13]. This is in line with retrospective data from the emergency department of the University Hospital Lund, Sweden, where the specificity of the cut-off of 14ng/l in chest pain patients aged 75 years or older was reported with 38%[14]. Several causes may contribute to the age-dependent increase of hsTnT: first, age per se is important. Concurrently, our data shows consistently higher hsTnT-levels in the old and very old subjects, even if they are free of known cardiac disease and cardiac remodelling in echocardiography. However, myocardial remodelling underlies early complex processes, before macroscopic morphology and function change[35–37]. Further, comorbidities associated with chronic myocardial injury increase by age and contribute to elevated hsTnT-values[38,39]. Not all such comorbidities might have been excluded even in the “super healthy” subgroup, particularly if they are more on subclinical levels. In patients with clinical suspicion of myocardial infarction and hsTnT-value above 14ng/l, current guidelines recommend a second hsTnT-determination after two hours to look for hsTnT-dynamics. Even if hsTnT-values do not further increase, an observational time of at least four hours in the emergency department entailing a third hsTnT-determination after 3 hours and an echocardiography is endorsed[1] before transfer to a cardiologic ward. Invasive coronary angiography is considered in case of high degree of clinical suspicion of myocardial infarction, while in patients with low-to-intermediate likelihood further non-invasive imaging is recommended by the ESC guidelines[1]. A recent collaborative analysis of three large diagnostic studies used the ESC algorithm and highlighted the consequences of decreasing specificity in higher age: 3,123 patients admitted for suspicion of acute myocardial infarction were prospectively enrolled. The percentage of patients aged 70 years or older remaining in the observe zone and requiring additional diagnostic testing was almost twice as high as in middle-aged (≥55 to < 70 years) and more than four times as high as in patients younger than 55 years[6]. Together, low specificity of the baseline rule-out value implies longer observational time in the emergency department, hospitalisation and

additional examinations for patients. Particularly the hazard of in the end unnecessary invasive coronary angiography is to consider owing to high risk of periprocedural events in elderly and multimorbid individuals [8]. Concerning the health system, long observation times and unnecessary diagnostics impair the workflow and resource management in emergency departments, which is recently more appreciated due to the current pandemic of coronavirus disease 2019 (COVID-19).

Previous studies[9,40,41] showed lower levels of high sensitivity troponins among women compared to men. As we report on hsTnT-distribution in an age group frequently seen in chest pain units and emergency departments[32], our results may provide an argument for sex specific thresholds. Indeed, the fourth universal definition of myocardial infarction[2] recommends the sex specific 99th percentile as upper reference limits for high sensitivity troponin assays. However, there is an on-going debate, whether sex-specific reference limits may improve prognosis in patients[42–44]. Our study encourages further analysis of hsTnT-levels in the population as well as in the emergency departments to advance clinical decision making with an improved accounting for sex differences and old age.

As age- or sex specific higher rule-out cut-off values barely improved the diagnostic performance of the ESC algorithm, but increased diagnostic complexity [6], the 2020 ESC guidelines continue to recommend uniform cut-off concentrations. At the same time, the importance of an integrative decision pathway based on full clinical assessment, electrocardiogram, hsTroponin-levels and non-invasive imaging was stressed[1]. To advance interpretation of the jigsaw piece “hsTnT” in clinical decision making, our study provides specificity data of the uniform rule-out cut-off value of 14ng/l as well as age-specific 99th percentiles of hsTnT for different strata (old versus very old age, sex, regular renal function, lack of cardiac disease history, regular left ventricular shape and function) in the mobile population aged 70 years or older.” The 2020 ESC guidelines limit the recommendation of uniform cut-off-concentrations, until further population-based and clinical data and information technology tools allow to calculate individual reference values based on age and comorbidities. We may report data from the first population-based study, which exclusively focusses on elderly individuals and comprises measurement of hsTnT as well as echocardiography. Our results may contribute to the necessary database comprising epidemiologic data for further meta-analyses and computation of individual risk. For this

purpose, we provide extensive data on hsTnT distribution overall and in a variety of strata for this focus group that is the most prevalent in emergency decision making.

Limitations:

The response proportion of the AugUR-study was 20.1% percent. It is similar to other recently established studies, even when they focused on more moderately aged adults[45]. By our design and recruitment strategy, there is a selection towards healthier subjects: our participants had to be mentally and physically fit enough to travel to the study centre and to answer all interview questions personally. This is mirrored by the fact, that 56.5% of non-participating subjects, who specified their reason for non-participation, declared, that they felt too ill to participate. Therefore, our participants do not represent the full old aged population, but reflect the "mobile" population aged above 70 years. For the aims of these analyses, this selection is advantageous, as we were interested in the relatively healthy old aged. Our data from medical exams including cardiac ultrasound, detailed medication intake history, and biomarker assessment enabled a further restriction to "healthy" old aged sub-cohorts.

We analysed the specificity of hsTnT under the assumption, that none of the AugUR participants had acute myocardial infarction by design. The current guideline definition of acute myocardial infarction entails cardiomyocyte necrosis in a clinical setting consistent with acute myocardial ischaemia[1]. The setting of our study did not at all correspond to acute myocardial infarction: the voluntary, mobile, elderly participants travelled on their own to the study side and were mentally as well as physically fit to go through the approximately two hours of study program without substantial exhaustion. None reported on specific symptoms during the study visit. It is naturally in the nature of myocardial ischaemia, that a study participant could have nevertheless suffered from silent infarction during the study visit. However, given the fact that 30% of participants had hsTnT-values above 14ng/l, a relevant bias of our data due to the rare event of acute, silent infarction during the study visit is not plausible.

Only 26 participants were 90 years of age or older. Therefore, estimates in the very old, particularly when further restricting to healthy subgroups, are subject to uncertainty by sparse numbers. Still, this pertains also to other studies.

Concerning the echocardiographic measurements, our study lacks three-dimensional data acquisition. Consequently, left ventricular mass was determined by the linear method using two-dimensional guided M-Mode in the parasternal long axis view, which relies on

assumptions of standardised left ventricular geometry and might be inaccurate in abnormally shaped ventricles and localised hypertrophy. However, the current guidelines of the European Association of Cardiovascular Imaging still explicitly recommend the linear method for large population studies[20,46].

Conclusion

In the elderly population aged at least 70 years, hsTnT-levels continue to raise with age, whilst sex and renal dysfunction are further relevant strata for hsTnT-concentrations in the elderly. The specificity of the 14ng/l cut-off hsTnT-value is substantially lower than 99%, even in healthy subjects. Our study data emphasize the need of further data and discussions on age-dependent cut-off values and also, within high age-groups, cut-off levels that reflect sex and kidney function.

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Author Contributions:

The following authors made substantial contributions to the conceptualisation or design:

investigation: AD, IMH, KJS, MEZ, CB

methodology: AD, IMH, AL, LSM, SW, RB, KJS, MEZ, CB

Data curation: AD, CB, SW, RB, IMH, KJS, MEZ

Formal analysis: AD, IMH, KJS, MEZ

Interpretation: AD, IMH, AL, LSM, KJS, MEZ

Funding acquisition: IMH, CB, AL, KJS

Supervision: IMH, AL, LSM, KJS

Validation: AD, CB, IMH, AL, LSM, KJS, MEZ

Writing (original draft preparation): AD

All authors contributed to the reviewing and editing of the manuscript.

Conflict of interest statement:

Roche Diagnostics has provided kits for assessment of hsTnT and NT-proBNP free of charge, but it did not play a role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

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Data sharing statement

Data are available upon reasonable request.

Figure captions:

Figure 1. Values of high-sensitivity troponin T in 1,129 participants of the AugUR study by age groups and sex

A box represents the lower (25%) and upper (75%) quartiles with median as a horizontal line within the box. Y-axis shows values on a log10-based scale. hsTnT: high-sensitivity troponin T.

Figure 2. Proportion below and above a high-sensitivity troponin T rule-out cut-off value of 14 ng/l in different AugUR subgroups.

The proportion of negatives according to the rule-out cut-off value of 14ng/l, who are correctly identified as not having acute myocardial infarction, decreases with sex, age and renal function (blue boxes), whilst the rate of false positives increases (orange boxes). Grey boxes represent the commonly accepted false positive rate of 1%.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. hsTnT: high-sensitivity troponin T. Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2). Subcohort II: additionally free of diabetes and obesity (body-mass index <30 kg/m^2) with a blood pressure <160/100mmHg at study visit. Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

Figure 3. Determinants of elevated high-sensitivity troponin T (>14ng/l)

Odds ratio estimates for high-sensitivity troponinT > 14 ng/l. Simple logistic regression without adjustment and after adjustment for age and sex. Presented are the OR and 95% CI. Dashed line indicates OR=1.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. LV: left ventricular. Elevated LV filling pressure: E/e' >14.

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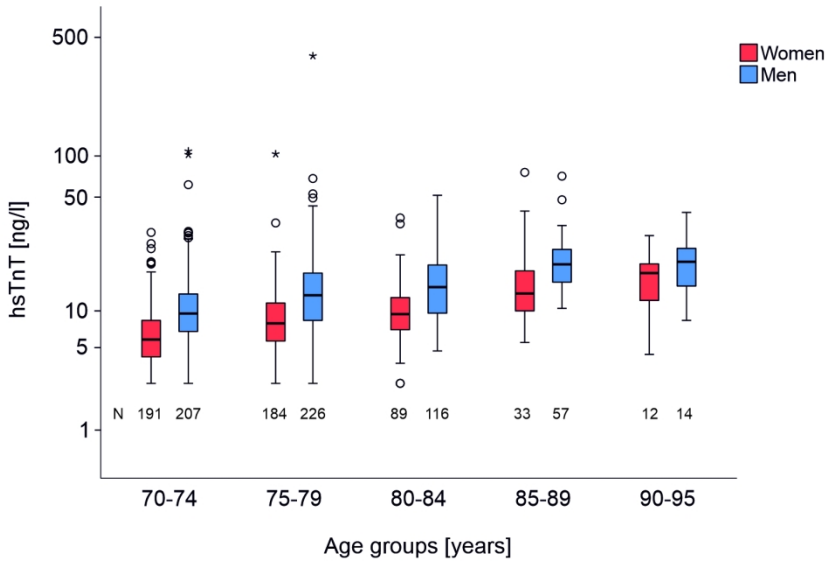

Figure 1. Values of high-sensitivity troponin T in 1,129 participants of the AugUR study by age groups and sex

A box represents the lower (25%) and upper (75%) quartiles with median as a horizontal line within the box. Y-axis shows values on a log10-based scale. hsTnT: high-sensitivity troponin T.

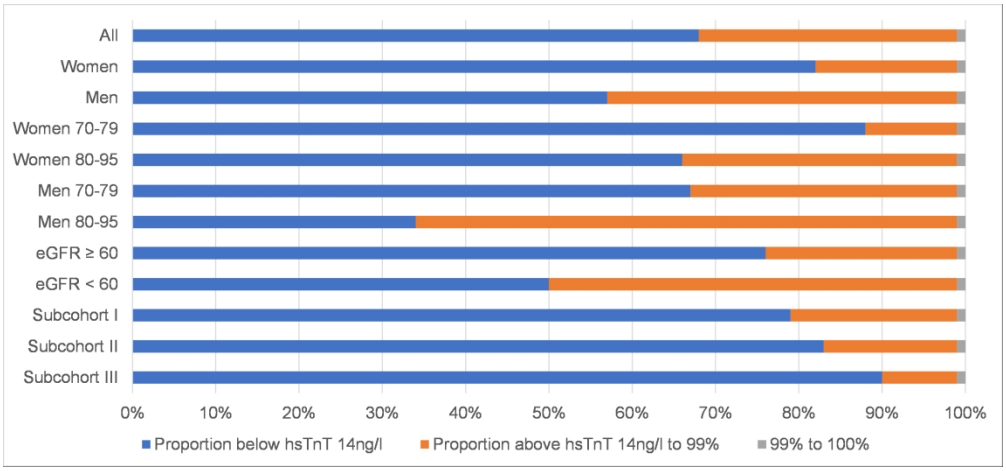

Figure 2. Proportion below and above a high-sensitivity troponin T rule-out cut-off value of 14 ng/l in different AugUR subgroups.

The proportion of negatives according to the rule-out cut-off value of 14ng/l, who are correctly identified as not having acute myocardial infarction, decreases with sex, age and renal function (blue boxes), whilst the rate of false positives increases (orange boxes). Grey boxes represent the commonly accepted false positive rate of 1%.

eGFRcrea glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m2. hsTnT: high-sensitivity troponin T. Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m2). Subcohort II: additionally free of diabetes and obesity (body-mass index <30 kg/m2) with a blood pressure <160/100mmHg at study visit. Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

641x296mm (118 x 118 DPI)

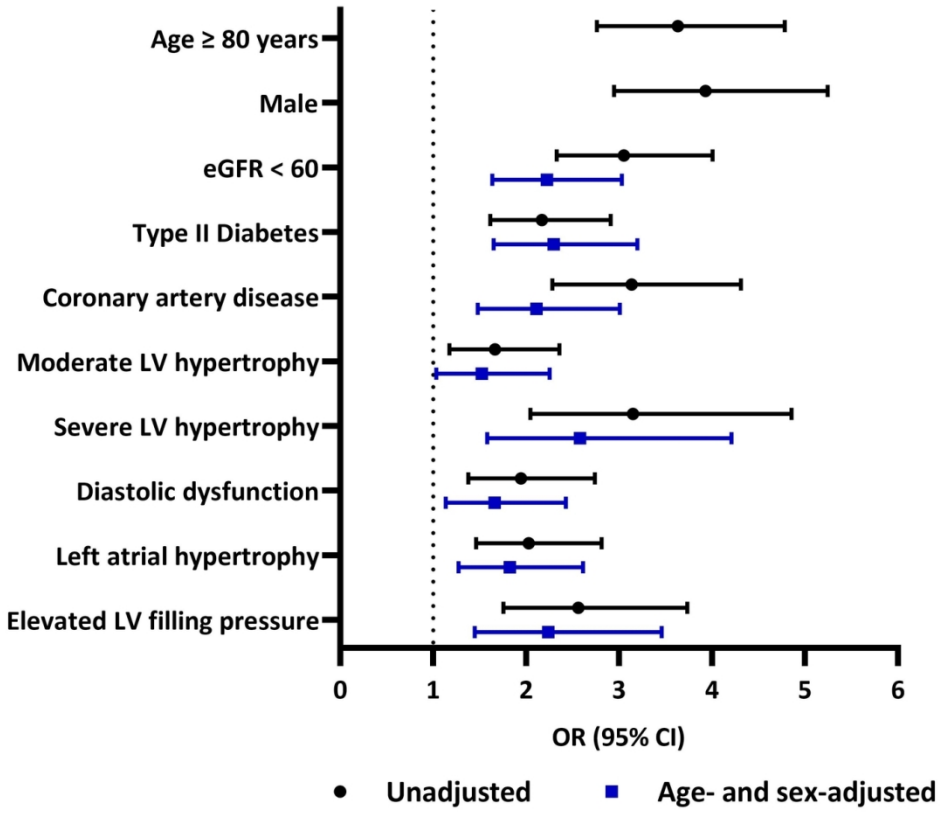

Figure 3. Determinants of elevated high-sensitivity troponin T (>14ng/l)
Odds ratio estimates for high-sensitivity troponinT > 14 ng/l. Simple logistic regression without adjustment and after adjustment for age and sex. Presented are the OR and 95% CI. Dashed line indicates OR=1. eGFRcrea glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m2. LV: left ventricular. Elevated LV filling pressure: E/e' >14.

125x110mm (300 x 300 DPI)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cross sectionalreporting guidelines, and cite them as:

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5

Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5
	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	22
Study size	#10	Explain how the study size was arrived at	5 and 8
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7 and 14-15
Statistical methods	#12c	Explain how missing data were addressed	n/a
Statistical methods	#12d	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	#12e	Describe any sensitivity analyses	7 and 14-15
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
Participants	#13b	Give reasons for non-participation at each stage	5 and 8

Participants	#13c	Consider use of a flow diagram	n/a
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	8 and 12
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 3 + caption
Main results	#16b	Report category boundaries when continuous variables were categorized	10,11,13,17, Fig3+caption
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9-17
Discussion			
Key results	#18	Summarise key results with reference to study objectives	18
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	22
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18-22
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18-22
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

Notes:

- 12b: 7 and 14-15
- 12e: 7 and 14-15
- 16a: Figure 3 + caption
- 16b: 10,11,13,17, Fig3+caption This checklist was completed on 02. April 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Distribution and specificity of high-sensitivity cardiac troponin T in older adults without acute cardiac conditions: cross-sectional results from the population-based AugUR study

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Distribution and specificity of high-sensitivity cardiac troponin T in older adults without acute cardiac conditions: cross-sectional results from the population-based AugUR study

Alexander Dietl[1], Martina E. Zimmermann[2], Caroline Brandl[2,3], Stefan Wallner[4], Ralph Burkhardt[4], Lars S. Maier[1], Andreas Luchner[5], Iris M. Heid[2], Klaus J. Stark[2]

[1] Department of Internal Medicine II, University Hospital Regensburg, Regensburg, Germany

[2] Department of Genetic Epidemiology, University of Regensburg, Regensburg, Germany

[3] Department of Ophthalmology, University Hospital Regensburg, Regensburg, Germany

[4] Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

[5] Department of Cardiology, Krankenhaus der Barmherzigen Brueder, Regensburg, Germany

Corresponding author:

Dr. Alexander Dietl

University Hospital Regensburg

Department of Internal Medicine II

D-93042 Regensburg

Germany

Email : alexander.dietl@ukr.de

Phone : +49 941 944 0

Fax : +49 941 944 5212

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Troponin, specificity, elderly, population-based, NSTEMI

Abstract

Objective: European guidelines recommended a uniform upper-reference limit of high sensitivity cardiac troponinT (hsTnT) to rule-out non-ST-segment-elevation myocardial infarction. Our study aimed to provide a hsTnT reference-distribution and to assess the specificity of the 14ng/l cut-off value in the mobile population ≥70years of age.

Design: A cross-sectional analysis was performed in the German AugUR study (**A**ltersbezogene **U**ntersuchungen zur **G**esundheit der **U**niversity of **R**egensburg).

Setting: Study population was the mobile population aged 70+ years living in the city and county of Regensburg, Germany.

Participants: A random sample was derived from the local population registries of residence. Of the 5,644 individuals invited, 1,133 participated (response ratio=20.1%). All participants came to the study centre and were mentally and physically mobile to conduct the protocol (face-to-face interview, blood draw, standardized transthoracic echocardiography). None of the participants was in an acute state of myocardial infarction.

Results: Among the 1,129 individuals with hsTnT measurements (overall median=10.0ng/l [25th, 75th percentile]=[7.0, 15.0ng/l]), hsTnT was higher among the older individuals and higher among men (men 70-74years median=9.6ng/l [7.2, 13.1ng/l]; men 90-95years median=21.2ng/l [14.6, 26.0ng/l]; women 70-74 years median=6.3ng/l [4.7, 8.7ng/l]; women 90-95years median=18.0ng/l [11.0, 21.0ng/l]). In participants with impaired kidney function ($eGFR_{crea}$<60ml/min/1.73m^2), hsTnT was elevated (median=13.6ng/l [9.4, 20.6ng/l]). Specificity of recommended upper-reference limit, 14ng/l, is 68%. Most false positives were among men aged >79years (specificity=34%). In a healthy subgroup (n=96, none of the following: overt heart disease, impaired renal function, blood pressure > 160/100mmHg, left ventricular hypertrophy, diastolic/systolic dysfunction), specificity was 90%.

Conclusion: In the elderly population without acute myocardial infarction, hsTnT further increases with age showing different levels for men and women. The specificity of the 14ng/l cut-off is considerably lower than 99%, even in healthy subjects.

Article Summary

Strengths and limitations of this study

Population-based approach: A major strength of our study is the population-based approach focussed on the age group, which is most often seen in chest pain units.

Appropriate design: The study was a-priori designed to determine reference values of biomarker incorporating thorough protocols for collection of serum and elaborated biobanking.

Rigorous conduct: The study protocol entailed firmly standardised procedures as well as the conduct by trained, experienced and quality-controlled staff.

Cardiac imaging: Echocardiography was performed according to current European and American guidelines following in advance defined standard operating procedures.

Focused on just one ethnic group: As the recruitment area in South-Eastern Germany implies a largely Caucasian population, we cannot report on high-sensitivity troponinT concentrations in further ethnicities.

Introduction

High sensitivity cardiac troponin T (hsTnT) is a sensitive marker of cardiomyocyte injury indicating myocardial damage resulting from, e.g. myocardial ischaemia, pulmonary embolism, myocarditis or Takotsubo syndrome[1–3]. In chest pain patients, hsTnT constitutes a mainstay for diagnosis of non-ST-segment elevation myocardial infarction. The 2020 Guidelines of the European Society of Cardiology for the management of acute coronary syndromes continue to recommend a uniform cut-off concentration of 14ng/l for rule-out of non-ST-elevation acute myocardial infarction in the 0/2-hour protocol. This hsTnT-value was initially derived from a pooled reference population of 616 subjects (mean age 44 years) and a study sample comprising 533 individuals (mean age 37 years), in which a value of 14ng/l signified approximately the 99th percentile of hsTnT-distribution [4,5]. In several further analyses, it turned out to be a sufficiently sensitive upper reference limit for rule-out of acute myocardial infarction in the emergency department [1,6,7].

While high sensitivity is crucial for a biomarker diagnosing an acute, life-threatening disease with immediate options for effective intervention, specificity can also be important: low specificity implies a large proportion of unnecessary examinations, hospitalization, and cardiac catherization along with risks of serious complications[6]. Older and multimorbid patients carry a particularly elevated risk for complications from percutaneous coronary intervention[8], which emphasizes the relevance of specificity particularly for the older adults. To this extent, large population-based studies have challenged uniform cut-off values due to considerable sex- and age-differences in hsTnT-distribution with decreasing specificity by age [9–11]. The dependency of hsTnT-concentrations on age implies major clinical impact: most chest pain patients are at advanced age [12] and the decreasing specificity of the uniform cut-off by age yields a growing number of false-positive results in the elderly [13,14]. Despite being the primary clinical target population for the application of these cut-off values, the elderly are less captured in published data on hsTnT-distribution [9–11]. This gap can be attributed to the specific needs of the elderly, which often hamper their participation in population-based studies or prompt general studies to exclude individuals above the age of, e.g., 70 years[15,16]. The aims of our analyses were to understand the distribution for hsTnT-values in the mobile population ≥70 years of age without acute cardiac disease and to quantify the specificity of the 14ng/l cut-off value at old age. We report on our cross-sectional data from

1,129 participants of the German AugUR study (**A**ltersbezogene **U**ntersuchungen zur **G**esundheit der **U**niversity of **R**egensburg), which focused on the mobile population ≥70years of age. The study protocol entailed a face-to-face interview, collection of serum samples and a standardized transthoracic echocardiography enabling a thorough assessment of even subtle subclinical cardiac disorders.

Methods

Study sample

The design of the German AugUR study has been described in detail previously[17]. Briefly, we recruited inhabitants at least 70 years of age in the city of Regensburg, Germany, and selected nearby counties. The local registries of residence provided a random sample of 5,971 subjects' postal addresses, who were invited by mail. Of these, (i) 327 persons were not contactable, as they had moved outside the study region or had meanwhile died, (ii) 3,187 persons did not respond, (iii) 1,324 responded negatively (i.e., declined participation by phone or in writing), and (iv) 1,133 participated (response among the 5,644 contactable =20.1%). For 402 non-participants, the specified reasons for denial were: 56.5% too ill, 6.2% no time, 20.1% no interest, 17.2% other. The 1,133 participants were able to come to the study centre at the University Medical Centre, to walk around independently, to answer all interview questions personally, and to conduct a two-hour study program including non-invasive medical exams. Thus, all participants had no acute cardiac events, were physically mobile and mentally fit. We consider our participants to reflect the "mobile" older population.

Patient and Public Involvement Statement

The AugUR survey is an epidemiologic, cross-sectional study, inviting a random sample of the general population aged 70 or more years. Accordingly, no specific group of patients is involved. Results are published in scientific journals and presented on the web page of the AugUR study (https://www.uni-regensburg.de/medizin/epidemiologie-praeventivmedizin/genetische-epidemiologie/augur/index.html). Specific results are accessible for every participant upon reasonable request.

Ethics statement

The study protocol, study procedures, and data protection strategy were all approved by the Ethics Committee of the University of Regensburg, Germany (vote 12-101-0258). All study

participants provided written consent after being informed about the study. The study was conducted according to the principles expressed in the Declaration of Helsinki. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

General data assessment

Sociodemographic factors, smoking behaviour, medication use and cardiovascular medical history (including existence, history, and time onset of cardiovascular diseases and interventions) were assessed in a standardised face-to-face interview by trained staff. Blood pressure was measured using an automatic device (Omron M10-IT; Omron Healthcare, Kyoto, Japan), pulse rate was determined by palpation after five minutes of resting time. Blood pressure was measured three times and the average of the second and third measurement were computed for further analyses.

Assessment of cardiac morphology and function by echocardiography

In order to assess even subclinical cardiac disorders, transthoracic echocardiography was performed using a commercially available ultrasound unit (HP Sonos 5500 with 2-4 MHz probe; Philips, Eindhoven, The Netherlands). The stored tracings were evaluated post hoc using analytical software Xcelera R3.2L1 Version 3.2.1.520 – 2011 (Philips Medical Systems, Amsterdam, Netherlands) as previously described[18]. The echocardiographic program focused on left atrial and ventricular morphology and function accounting for chamber-specific cardiac remodelling processes[19] according to the current guidelines[20]: left atrial volume was determined by two-dimensional volumetric measurement based on tracings of the blood-tissue interface in apical four-chamber view. M-Mode measurements for calculating left ventricular mass were obtained from parasternal long axis view and determined perpendicular to the left ventricular axis. Left ventricular mass was computed by the Devereux formula[21]. Left atrial volume and left ventricular mass were indexed to body-surface area approximated by DuBois' formula[22]. To estimate left ventricular filling pressures, the ratio

of the transmitral early peak velocity by pulsed wave Doppler (E) over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler (mean e') was determined (E/mean e'). Left ventricular diastolic dysfunction was evaluated according to recent recommendations[23]. Systolic function was assessed as ejection fraction estimated by the modified Simpson's method[20] based on monoplanar measurements in the apical four chamber view. Each of the measurements used for further analyses was repeated three times for regular rhythm and ten times in case of arrhythmia to reduce random error.

High-sensitivity Troponin T and NT-proBNP measurements

Collection and procession of biosamples were conducted following standard operation procedures developed for this study based on established methods and recommendations[24]. Deviations from these standard operation procedures (e.g., extended sample handling at room temperature) were recorded and linked with the biosample information. All samples were processed immediately and kept on dry ice before final storage at the end of the day. Identification, assignment and link to electronic case report form (eCRF) data for biosamples including 2D-barcoded tubes were managed by self-developed integrated software.

Non-fasting blood samples were drawn in a sitting position after at least five minutes of resting. Mild venous stasis was applied for a maximum duration of one minute. Whole blood was taken using a 21G multifly needle. Two samples were used for ad hoc analysis. Serum tubes with clot activator were left in upright position for 30 minutes after blood draw and were centrifuged at 2,000 g for 15 minutes at room temperature to separate serum from the cellular fraction as soon as possible. Supernatants from serum tubes were transferred to 2D-barcoded tubes for storage at -80°C.

Measurements for hsTnT and N-terminal prohormone B-type (brain) natriuretic peptide (NT-proBNP) were conducted in stored serum samples by the Department of Clinical Chemistry and Laboratory Medicine of the University Hospital Regensburg on a cobas e411 (Roche Diagnostics, Rotkreuz, Switzerland). After measurement, data were exported from SWISSLAB (NEXUS SWISSLAB GmbH, Berlin, Germany) in Excel format and processed with Microsoft Access 2019 (Microsoft Corporation, Redmond, WA, USA), SAS 9.4 (SAS Institute Inc., Cary,

NC, USA) and SPSS 25.0.0.2 (IBM Corporation, Armonk, NY, USA.). 31 values for hsTnT [ng/l or pg/ml] were on the lower detection limit of "<3" ng/l. Those results were winsorised to "2.9" to discriminate from true "3.0". For NT-proBNP [pg/ml], no values with extremes beyond specified measurement range (5-35,000 pg/ml) were detected.

Statistical methods

Continuous variables are reported as mean and standard deviation (SD) or as median with the 25th and 75th percentiles. Estimates of confidence intervals for 99th and 95th percentiles were derived by bootstrap analysis using bias corrected and accelerated intervals. Categorical variables are reported as proportions. Odds ratio estimates for hsTnT-values > versus <= 14ng/l were computed by simple logistic regression for each of the covariates separately: age, male sex, impaired kidney function, type II diabetes, history of coronary artery disease, left ventricular hypertrophy, diastolic dysfunction, left atrial hypertrophy and elevated filling pressure (defined as E/e'>14). This was repeated adjusting for age and sex, as applicable. We used the STROBE cross sectional checklist when writing our report[25]. All analyses were carried out with SPSS 25.0.0.2 (IBM, Armonk, USA).

Results

Characteristics of the study sample

1,129 participants out of 1,133 showed valid hsTnT-values and were included for further analyses. Age ranged from 70.3 to 95.0 years, with a median of 76.7 years (25th, 75th-percentile = 73.7, 80.9 years). Demographic, clinical and laboratory characteristics are shown in **Table 1**. Of note, all individuals came walking to the study centre at the University medical centre, participated in the two-hour study program with little exhaustion mentally or physically and can thus be considered mobile elderly. None of the participants had any sign of acute cardiac condition, particularly myocardial infarction, throughout the study visit. While our participants were all relatively healthy by design, they included medical conditions to the extent as one expects from the mobile population of that age.

Characteristics	Women (n=509)	Men (n=620)
Age [years]	77.34 ± 5.02	77.88 ± 5.06
Body-mass index [kg/m^2]	27.8 ± 5.0	28.2 ± 4.0
Diabetes [%]	19.4	23.2
Hypertension [%]	74.2	72.9
Coronary artery disease [%]	9.8	23.1
Heart failure [%]	16.0	13.5
Tobacco use (present/past) [%]	25.5	60.3
$eGFR_{crea}$ [ml/min/1.73m^2]	68.5 ± 16.2	66.1 ± 16.4

Table 1: Baseline characteristics of the study sample

Shown are mean and standard deviation or proportions for the 1,129 subjects separately for women and men.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Distribution of hsTnT-values by age, sex and glomerular filtration rate

First, we looked at the distribution of hsTnT levels by age groups and sex **(Figure 1)**. HsTnT-values increased with age and were higher in men than in women (**Table 2**). Further, we report

on values separately for normal and reduced glomerular filtration rate (eGFR≥ vs. < 60ml/min per 1.73m^2, derived from serum creatinine, **Table 3**).

For actual diagnosis of acute non-ST-elevation myocardial infarction in symptomatic patients, the 2020 Guidelines of the European Society of Cardiology endorse a rule-in hsTnT-cut-off concentration of 52ng/l, which implies immediate referral of chest pain patients to invasive diagnostics[1]. In 13 subjects (1,2%) of our study, hsTnT was measured above this rule-in cut-off (≥ 52ng/l) with a median of 72.1ng/l (55.4, 102.3ng/l) and a maximum of 421ng/l.

Specificity of the rule-out upper reference limit (14ng/l) in the mobile elderly

Next, we intended to estimate the specificity of the endorsed rule-out upper reference limit of hsTnT [1] in our mobile elderly individuals considered free of acute myocardial infarction **(Figure 2).** Applying the recommended cut-off value of 14ng/l, 70% (790/1,129 subjects) of our study participants were below this cut-off. Main determinants of hsTnT-values above 14ng/l were age, male sex, impaired kidney function, type II diabetes, history of coronary artery disease, left ventricular hypertrophy, diastolic dysfunction, left atrial hypertrophy and elevated filling pressure (E/e'>14, **Figure 3**). As this cut-off was defined as the 99th percentile of reference samples without acute myocardial infarction in the attempt to yield 99% specificity [1,4,5], this is in line with the notion that, among our study participants, 70% were correctly identified (true negative for acute myocardial infarction), but 30% (339/1,129) were not (false-positive). These 339 individuals showed a median level of 19.4ng/l (15.6, 24.9ng/l). They were older, more likely men and more likely with diabetes, stable coronary artery disease, heart failure or impaired kidney function than the 790 individuals below the cut-off (**Table 4**). Regarding echocardiographic measurements, elevated left ventricular mass was detected.

Further stratification revealed a particularly low specificity for the 14ng/l hsTnT level in men (57%) as well as in subjects with impaired kidney function (50% for eGFR < 60 ml/min/1.73m^2) and bottommost in men aged 80 years or older (34%, **Table 5**).

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
Women n	191	184	89	33	12	509
Mean ± SD	7.54 ± 4.56	9.76 ± 8.51	11.03 ± 6.32	16.66 ± 13.62	17.02 ± 6.97	9.77 ± 7.75
Minimum	2.9	2.9	2.9	6.0	4.9	2.9
5th percentile	2.9	2.9	4.5	6.2	4.9	3.1
10th percentile	3.3	3.6	5.2	6.9	6.4	4.0
25th percentile	4.7	6.1	7.2	9.7	11.0	5.7
Median	6.3	8.2	9.5	13.1	18.0	8.0
75th percentile	8.7	11.3	13.0	18.9	21.0	11.5
90th percentile	13.5	15.1	18.7	27.8	28.7	17.4
95th percentile	16.1	19.7	22.5	55.1	-	21.5
Maximum	32.8	102.8	40.6	78.8	31.3	102.8
Men n	207	226	116	57	14	620
Mean ± SD	11.96 ± 11.49	16.31 ± 28.60	16.71 ± 9.87	21.89 ± 10.31	21.16 ± 8.98	15.56 ± 19.49
Minimum	2.9	2.9	5.2	10.4	8.6	2.9
5th percentile	4.8	5.2	6.6	11.3	8.6	5.3
10th percentile	5.6	6.2	7.5	12.5	9.4	6.1
25th percentile	7.2	8.6	9.6	15.5	14.6	8.3
Median	9.6	12.8	14.5	20.4	21.2	12.3
75th percentile	13.1	18.0	20.3	25.6	26.0	18.1
90th percentile	18.4	24.5	30.2	30.2	36.1	26.4
95th percentile	28.4	30.4	37.7	38.0	-	31.3
Maximum	107.1	421.3	56.5	74.6	44.0	421.3

Table 2: hsTroponinT values [ng/l] by age groups and sex in 1,129 participants of the AugUR stud

Age groups	70 - 74	75 - 79	80 - 84	85 - 89	90 - 95	All (70 - 95)
$eGFR_{crea}$ ≥60 n	322	298	113	35	10	778
Mean ± SD	9.16 ± 7.68	10.93 ± 7.79	12.31 ± 6.42	17.31 ± 7.29	14.91 ± 14.75	10.74 ± 7.74
Minimum	2.9	2.9	2.9	6.3	4.9	2.9
5th percentile	3.1	3.1	5.2	6.5	4.9	3.2
10th percentile	4.0	4.7	5.8	8.3	5.3	4.5
25th percentile	5.5	6.9	7.9	11.1	9.8	6.3
Median	7.4	9.2	10.7	16.8	14.8	8.9
75th percentile	10.4	13.2	15.3	22.4	17.8	13.2
90th percentile	14.7	18.8	21.4	28.6	27.5	18.5
95th percentile	21.0	22.6	24.7	29.7	-	23.5
Maximum	107.1	102.8	40.6	33.1	28.2	107.1
$eGFR_{crea}$ <60 n	70	108	90	54	16	338
Mean ± SD	13.07 ± 13.85	20.38 ± 40.60	16.55 ± 10.98	21.95 ± 13.74	21.96 ± 8.18	18.17 ± 25.25
Minimum	2.9	3.4	4.7	7.3	9.8	2.9
5th percentile	4.1	4.8	5.5	9.9	9.8	5.3
10th percentile	5.3	6.9	6.9	11.8	10.0	6.8
25th percentile	7.0	9.7	9.1	13.5	19.4	9.4
Median	10.0	13.5	12.2	19.0	21.2	13.6
75th percentile	13.7	19.7	20.8	23.7	25.8	20.6
90th percentile	20.8	29.0	32.1	33.7	35.1	29.3
95th percentile	30.2	47.4	41.3	58.3	-	43.6
Maximum	101.8	421.3	56.5	78.8	44.0	421.3

Table 3: hsTroponinT values [ng/l] by age groups and $eGFR_{crea}$ categories in 1,129 participants of the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m²; a value of 60 was used to determine between good and limited kidney function

hsTroponinT [ng/l]	<14ng/l	n	≥ 14 ng/l	n
Age [years]	76.5 ± 4.3	790	80.3 ± 5.6	339
Female [%]	54.4	790	23.3	339
Body-mass index [kg/m^2]	27.7 ± 4.3	790	28.8 ± 4.9	335
Diabetes [%]	17.3	790	31.3	339
Hypertension [%]	72.1	788	76.6	338
BP <160/100mmHg [%]	92.0	789	89.7	339
Tobacco use (present/past) [%]	41.0	790	53.1	339
Low-density lipoprotein [mg/dl]	148.2 ± 33.8	701	139.1 ± 34.2	285
Coronary artery disease (self-reported) [%]	11.8	790	29.6	338
Heart failure (self-reported) [%]	11.5	788	21.7	336
$eGFR_{crea}$ [ml/min/1.73m^2]	70.4 ± 14.04	779	59.5 ± 17.2	337
NT-proBNP [pg/ml]	265.6 ± 355.5	790	963.3 ± 2349.0	339
Heart rate [beats per minute]	69.0 ± 11.0		67.8 ± 11.8	338
Regular rhythm [%]	93.0	599	81.0	248
LVMi [g/m^2]	103.6 ± 28.1	472	121.4 ± 36.31	179
Left atrial volume/BSA [ml/m^2]	37.5 ± 14.0	575	44.5 ± 18.1	235
E/mean e'	11.1 ± 3.4	530	12.3 ± 4.5	210
Diastolic dysfunction [%]	60.6	563	74.9	227
Ejection fraction [%]	60.7 ± 6.9	582	58.9 ± 9.2	237

Table 4: Characteristics of the study sample divided by the recommended rule-out cut-off of high-sensitivity troponin T for non-ST-segment elevation myocardial infarction in case of no relevant increase within 2 hours (14ng/l)

Shown are mean and standard deviation or proportions.

BP: blood pressure. $eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine. NT-proBNP: N-terminal prohormone of brain natriuretic peptide. LVMi: ratio of left ventricular mass to body surface area. BSA: body surface area. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. Diastolic dysfunction determined according to[23].

	n	99th hsTnT percentile [95% CI]	95th hsTnT percentile [95% CI]	Proportion below hsTnT 14ng/l
All	1,129	54 [44 - 74]	29 [26 – 31]	68
Stratified by sex				
Women	509	38 [27 - 79]	22 [20 - 23]	82
Men	620	64 [46 - 102]*†	31 [29 - 36]*	57
Stratified by sex and age				
Women 70-79 yrs	375	29 [23 - 58]	19 [15 - 21]	88
Women 80-95 yrs	134	67 [39 - 79]	27 [22 - 39]	66
Men 70-79 yrs	433	70 [42 - 281]*‡	30 [26 - 33]*¶	67
Men 80-95 yrs	187	59 [52 - 75]	37 [31 - 46]	34
Stratified by kidney function				
eGFR ≥ 60	778	33 [30 - 36]	24 [22 - 26]	76
eGFR < 60	338	77 [56 - 308]*§	44 [34 - 53]*Δ	50
Subcohort I				
All	618	32 [28 - 33]	22 [21 - 25]	79
Stratified by sex				
Women	289	25 [21 - 41]	17 [15 - 20]	90
Men	329	32 [30 - 33]	25 [23 - 28]	70
Stratified by age group				
70-79 yrs	507	30 [26 - 33]	21 [19 - 23]	83
80-95 yrs	111	40 [31 - 41]	28 [23 - 32]	62
Subcohort II				
All	366	31 [26 - 33]	20 [17 - 23]	83
Stratified by sex				
Women	173	22 [21 - 22]	16 [14 - 20]	90
Men	193	33 [31 - 33]	25 [18 - 29]	77
Stratified by age group				
70-79 yrs	304	30 [22 - 33]	18 [15 - 21]	88
80-95 yrs	62	N/A	29 [22 - 33]	60
Subcohort III				
All	96	N/A	17 [14 - 25]	90
Stratified by sex				
Women	49	N/A	17 [11 - 20]	94
Men	47	N/A	23 [14 - 29]	85
Stratified by age group				
70-79 yrs	86	N/A	14 [12 - 20]	94
80-95 yrs	10	N/A	N/A	50

Table 5: The 99th and 95th percentiles of high-sensitivity troponin T and percentiles corresponding to the recommended rule-out cut-off for non-ST-segment elevation myocardial infarction (14ng/l).

Shown are 99th and 95th percentiles with 95% confidence intervals in the entire AugUR study sample **(all)** with further stratification for sex, age and renal function, as well as in subcohorts free of overt heart disease and impaired renal function **(subcohort 1)**, comorbidities associated with elevated hsTroponinT (diabetes, obesity; **subcohort 2**) and subtle cardiovascular disease measurable by echocardiography **(subcohort 3)**.

Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2).

Subcohort II: as subcohort I, additionally free of diabetes and obesity (body-mass index <30 kg/m^2) with a blood pressure <160/100mmHg at study visit.

Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

*Leave-one-out analyses revealed an influential observation: one man (age 77years, eGFR 59ml/min/1.73, no coronary artery disease, LVMi 117g/m2, EF 65%) exhibited an extraordinarily elevated hsTnT-level of 421ng/l. Excluding it, percentiles and 95% confidence intervals were lowered to †57 [46 - 75], ‡63 [38 - 101] and §74 [55 - 93] for the 99th percentiles in ng/l [95%CI] and 31 [30 - 33], ¶ 29 [26 – 33] and $^{\Delta}$43 [33 – 49] for the 95th percentiles in ng/l [95%CI].

Left ventricular hypertrophy: left ventricular mass to body surface area >115g/m2 for men / 95g/m2 for women. E/e': ratio of the transmitral early peak velocity by pulsed wave Doppler over mean early diastolic velocity determined at the septal and lateral mitral annulus by tissue Doppler. EF: ejection fraction. $eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Overall, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction was below 99%, ranging from 34% to 88% in different sex-, age-, and eGFR-groups.

Specificity of the rule-out upper reference limit (14ng/l) in healthy subgroups

Next, we evaluated the specificity of 14ng/l hsTnT cut-off value in a healthy subgroup of our study participants **(Table 5)**: in subjects free of clinical coronary artery disease, heart failure or impaired renal function (subcohort I, n=618) specificity increased to 79% compared to the 68% in all participants. This proportion barely changed by additional exclusion of diabetic and obese participants as well as subjects with a measured blood pressure >160/100mmHg (83%; subcohort II, n=366). To further account for subtle, asymptomatic cardiac disorders, echocardiographic data was used to finally analyse a subgroup additionally free of any of the following: (i) no left ventricular hypertrophy (left ventricular mass to body surface area >115g/m^2 for men; 95g/m^2 for women)[20], (ii) no elevated left ventricular filling pressure (E/mean e' > 14)[23] and (iii) no left ventricular systolic dysfunction (ejection fraction < 50%)[26]. In the resulting subgroup (subgroup III, n=96), specificity increased to 90%, whilst remaining poor in participants above 79 years of age (50%).

Together, the specificity of the endorsed rule-out cut-off hsTnT-value for acute non-ST-segment elevation myocardial infarction ranged between 79% to 90% in the healthy subgroups.

Upper percentiles in the elderly

As results of the low specificity corresponding to 14ng/l hsTnT in our study participants, we were interested, which value of hsTnT reflected the 99th and 95th percentiles in our elderly individuals. The 99th percentile of the entire study sample was 54ng/l, showing higher levels in men and impaired kidney function (**Table 5**). Excluding overt cardiac disease and renal dysfunction (subcohort I), the 99th percentile was considerably lower (32ng/l). Further

exclusion of diabetes, obesity and elevated blood pressure (>160/100mmHg, subcohort II) did only slightly lower the 99th percentile (31 ng/l).

Since age, sex and kidney function defined relevant strata for hsTnT levels throughout our analyses and are usually known parameters in the setting of hospital admission for suspected myocardial infarction, we provide our 95th percentile values in the corresponding subcohorts and separately by these strata **(Table 6)**.

hsTroponinT [ng/l]	Women				Men			
Age	**70 - 79**		**80 - 95**		**70 - 79**		**80 - 95**	
	95th percentile	n	95th percentile	n	95th percentile	n	95th percentile	n
eGFR ≥ 60	17.4	293	22.6	66	24.4	327	29.2	92
eGFR < 60	21.6	75	35.1	66	57.0	103	47.7	94

Table 6: Upper limit (95th percentile) of blood ranges for high-sensitivity troponin T in the AugUR study

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine [ml/min/1.73m^2].

Discussion

In our study sample comprising 1,129 mobile, elderly participants free from symptoms of acute myocardial infarction, hsTnT-levels increase with age, are considerably higher in men than in women and rise in participants with impaired renal function. The specificity of the endorsed rule-out upper reference limit of hsTnT (14ng/l) is just 70% in the entire study sample, while the cut-off from guidelines was set to reflect 99% specificity[1,4,5]. A particularly low specificity, at 34%, is found among men aged 80 years or older. Correspondingly, all 99th percentiles in our entire study sample as well as in healthy subcohorts are substantially above the cut-off of 14ng/l. Finally, we provide hsTnT-values reflecting a specificity of 95% in our study stratified for sex, age and kidney function to supply physicians with an estimate of specificity in their ageing patients.

Distribution of hsTnT in the elderly

hsTnT-assay was established in healthy study samples a decade ago [4,5]. The 99th percentile of the hsTnT-distribution gained soon major interest, as it turned out to be a sufficient upper reference limit for rule-out of acute myocardial infarction in numerous further analyses [1,6,7]. One of the first studies assessing the hsTnT-assay reported an estimated 99th percentile of 13.5ng/l in a pooled reference population of 616 subjects with mean age of 44 years and age ranging from 20 to 71 years [4]. A second study sample comprised 533 participants with a mean age of 37 years including 1 subject older than 70 years and reported a 99th percentile of 14.2ng/l [5]. However, a joint analyses of data from large, population-based studies including the Dallas Heart Study (DHS), the Atherosclerosis Risk in Communities Study (ARIC) and the Cardiovascular Health Study challenged uniform cut-off values, as the authors reported considerable sex- and age-differences for 99th percentile values[9]. Accordingly, in the Generation Scotland Scottish Family Health Study (GS:SFHS) entailing 19,501 individuals, the 14ng/l-value showed a good fit in age groups below 60 years, whereas the 99th percentile is about 3-fold higher in participants above 60 years of age [10,11]. The increasing hsTnT levels in the age groups beyond 60 years are of particular clinical interest, as they correspond to the median age of patients suffering from troponin positive myocardial infarction in emergency departments, e.g. 70 years (58.1, 78.0 years) in the German chest pain

unit registry [12]. Nevertheless, the published data on hsTnT-distribution in the elderly is scarce and hitherto derived from population-based studies, in which recruitment of younger participants prevailed by far as in DHS, ARIC and GS:SFHS [9–11]. Thus, our study complements the discussed published data by focusing on the very old (median age 76.7 years [73.7, 80.9 years], age ranging from 70 to 95years) and provides relevant evidence for estimating the hsTnT distribution in the elderly: the recommended rule-out upper reference limit of hsTnT (14ng/l) is just the 70th percentile in our entire study sample of 1,129 individuals and is particularly low, at the 34th percentile, among men aged 80 years or older. The 99th percentile in our entire study sample is four-fold higher than 14ng/l.

Indeed, these values have to be interpreted with caution, as several illnesses with increasing age-dependent prevalence are *per se* associated with elevated hsTnT-levels, e.g. impaired kidney function, obesity, diabetes mellitus type II and irregular heart rhythm [7,10,27]. Furthermore, elevated hsTnT-levels are linked to elevated blood pressure[28,29] as well as signs of subtle, non-overt cardiac disease with increasing prevalence in the elderly, as increased left ventricular filling pressure [30] and left ventricular hypertrophy[31]. However, even in our reasonably healthy sub-cohort free of pre-existing cardiac disease, i.e., free of all discussed comorbidities and having blood pressure below 160/100mmHg, the 99th percentile is calculated as 31ng/l and thus more than twice as high as the recommended rule-out cut-off value of 14ng/l. In the very healthy sub-cohort, that is additionally free of echocardiographic signs of non-overt heart disease, specificity of the 14ng/l cut-off value is down to 90%.

The effect of age and sex on cut-off specificity is not only clear for hsTnT: Welsh and colleagues[10] compared cardiac troponin T and I in a large general population cohort. Despite the fact, that cardiac troponin T and I are only weakly correlated with each other and show different extent of association with cardiovascular risk factors, the 99th percentiles differ between men and women beyond the age of 70 years for both biomarkers[10].

Clinical implications

In chest pain patients, elevated age and comorbidities are highly prevalent, as depicted by the German chest pain unit registry[32]. Both are associated with increased risk of coronary artery disease and entail a raising incidence of non-ST-segment elevation myocardial

infarction[6,33]. High sensitivity is evidently crucial for a biomarker diagnosing an acute, life-threatening disease: missed acute cardiac ischemia is associated with considerable mortality [34]. Thus, whereas low sensitivity of the hsTnT-rule-out cut-off value implies elevated mortality, ramifications of low specificity are less obvious: even in the absence of acute myocardial infarction, age and comorbidities as well as elevated hsTnT-values are frequent in chest pain unit patients [32]: retrospective analyses of 3,219 emergency patients reported 41.5% of subjects aged older than 69 years without acute coronary syndrome above the upper reference limit of 14ng/l[13]. This is in line with retrospective data from the emergency department of the University Hospital Lund, Sweden, where the specificity of the cut-off of 14ng/l in chest pain patients aged 75 years or older was reported with 38%[14]. Several causes may contribute to the age-dependent increase of hsTnT: first, age per se is important. Concurrently, our data shows consistently higher hsTnT-levels in the old and very old subjects, even if they are free of known cardiac disease and cardiac remodelling in echocardiography. However, myocardial remodelling underlies early complex processes, before macroscopic morphology and function change[35–37]. Further, comorbidities associated with chronic myocardial injury increase by age and contribute to elevated hsTnT-values[38,39]. Not all such comorbidities might have been excluded even in the “super healthy” subgroup, particularly if they are more on subclinical levels. In patients with clinical suspicion of myocardial infarction and hsTnT-value above 14ng/l, current guidelines recommend a second hsTnT-determination after two hours to look for hsTnT-dynamics. Even if hsTnT-values do not further increase, an observational time of at least four hours in the emergency department entailing a third hsTnT-determination after 3 hours and an echocardiography is endorsed[1] before transfer to a cardiologic ward. Invasive coronary angiography is considered in case of high degree of clinical suspicion of myocardial infarction, while in patients with low-to-intermediate likelihood further non-invasive imaging is recommended by the ESC guidelines[1]. A recent collaborative analysis of three large diagnostic studies used the ESC algorithm and highlighted the consequences of decreasing specificity in higher age: 3,123 patients admitted for suspicion of acute myocardial infarction were prospectively enrolled. The percentage of patients aged 70 years or older remaining in the observe zone and requiring additional diagnostic testing was almost twice as high as in middle-aged (≥55 to < 70 years) and more than four times as high as in patients younger than 55 years[6]. Together, low specificity of the baseline rule-out value implies longer observational time in the emergency department, hospitalisation and

additional examinations for patients. Particularly the hazard of in the end unnecessary invasive coronary angiography is to consider owing to high risk of periprocedural events in elderly and multimorbid individuals [8]. Concerning the health system, long observation times and unnecessary diagnostics impair the workflow and resource management in emergency departments, which is recently more appreciated due to the current pandemic of coronavirus disease 2019 (COVID-19).

Previous studies[9,40,41] showed lower levels of high sensitivity troponins among women compared to men. As we report on hsTnT-distribution in an age group frequently seen in chest pain units and emergency departments[32], our results may provide an argument for sex specific thresholds. Indeed, the fourth universal definition of myocardial infarction[2] recommends the sex specific 99th percentile as upper reference limits for high sensitivity troponin assays. However, there is an on-going debate, whether sex-specific reference limits may improve prognosis in patients[42–44]. Our study encourages further analysis of hsTnT-levels in the population as well as in the emergency departments to advance clinical decision making with an improved accounting for sex differences and old age.

As age- or sex specific higher rule-out cut-off values barely improved the diagnostic performance of the ESC algorithm, but increased diagnostic complexity [6], the 2020 ESC guidelines continue to recommend uniform cut-off concentrations. At the same time, the importance of an integrative decision pathway based on full clinical assessment, electrocardiogram, hsTroponin-levels and non-invasive imaging was stressed[1]. To advance interpretation of the jigsaw piece “hsTnT” in clinical decision making, our study provides specificity data of the uniform rule-out cut-off value of 14ng/l as well as age-specific 99th percentiles of hsTnT for different strata (old versus very old age, sex, regular renal function, lack of cardiac disease history, regular left ventricular shape and function) in the mobile population aged 70 years or older.” The 2020 ESC guidelines limit the recommendation of uniform cut-off-concentrations, until further population-based and clinical data and information technology tools allow to calculate individual reference values based on age and comorbidities. We may report data from the first population-based study, which exclusively focusses on elderly individuals and comprises measurement of hsTnT as well as echocardiography. Our results may contribute to the necessary database comprising epidemiologic data for further meta-analyses and computation of individual risk. For this

purpose, we provide extensive data on hsTnT distribution overall and in a variety of strata for this focus group that is the most prevalent in emergency decision making.

Limitations:

The response proportion of the AugUR-study was 20.1% percent. It is similar to other recently established studies, even when they focused on more moderately aged adults[45]. By our design and recruitment strategy, there is a selection towards healthier subjects: our participants had to be mentally and physically fit enough to travel to the study centre and to answer all interview questions personally. This is mirrored by the fact, that 56.5% of non-participating subjects, who specified their reason for non-participation, declared, that they felt too ill to participate. Therefore, our participants do not represent the full older population, but reflect the "mobile" population aged above 70 years. For the aims of these analyses, this selection is advantageous, as we were interested in the relatively healthy older adults. Our data from medical exams including cardiac ultrasound, detailed medication intake history, and biomarker assessment enabled a further restriction to "healthy" older sub-cohorts.

We analysed the specificity of hsTnT under the assumption, that none of the AugUR participants had acute myocardial infarction by design. The current guideline definition of acute myocardial infarction entails cardiomyocyte necrosis in a clinical setting consistent with acute myocardial ischaemia[1]. The setting of our study did not at all correspond to acute myocardial infarction: the voluntary, mobile, elderly participants travelled on their own to the study side and were mentally as well as physically fit to go through the approximately two hours of study program without substantial exhaustion. None reported on specific symptoms during the study visit. It is naturally in the nature of myocardial ischaemia, that a study participant could have nevertheless suffered from silent infarction during the study visit. However, given the fact that 30% of participants had hsTnT-values above 14ng/l, a relevant bias of our data due to the rare event of acute, silent infarction during the study visit is not plausible.

Only 26 participants were 90 years of age or older. Therefore, estimates in the very old, particularly when further restricting to healthy subgroups, are subject to uncertainty by sparse numbers. Still, this pertains also to other studies.

Concerning the echocardiographic measurements, our study lacks three-dimensional data acquisition. Consequently, left ventricular mass was determined by the linear method using two-dimensional guided M-Mode in the parasternal long axis view, which relies on assumptions of standardised left ventricular geometry and might be inaccurate in abnormally

shaped ventricles and localised hypertrophy. However, the current guidelines of the European Association of Cardiovascular Imaging still explicitly recommend the linear method for large population studies[20,46].

Conclusion

In the elderly population aged at least 70 years, hsTnT-levels continue to raise with age, whilst sex and renal dysfunction are further relevant strata for hsTnT-concentrations in the elderly. The specificity of the 14ng/l cut-off hsTnT-value is substantially lower than 99%, even in healthy subjects. Our study data emphasize the need of further data and discussions on age-dependent cut-off values and also, within high age-groups, cut-off levels that reflect sex and kidney function.

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Author Contributions:

The following authors made substantial contributions to the conceptualisation or design:

investigation: AD, IMH, KJS, MEZ, CB

methodology: AD, IMH, AL, LSM, SW, RB, KJS, MEZ, CB

Data curation: AD, CB, SW, RB, IMH, KJS, MEZ

Formal analysis: AD, IMH, KJS, MEZ

Interpretation: AD, IMH, AL, LSM, KJS, MEZ

Funding acquisition: IMH, CB, AL, KJS

Supervision: IMH, AL, LSM, KJS

Validation: AD, CB, IMH, AL, LSM, KJS, MEZ

Writing (original draft preparation): AD

All authors contributed to the reviewing and editing of the manuscript.

Conflict of interest statement:

Roche Diagnostics has provided kits for assessment of hsTnT and NT-proBNP free of charge, but it did not play a role in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

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Data sharing statement

Data are available upon reasonable request.

Figure captions:

Figure 1. Values of high-sensitivity troponin T in 1,129 participants of the AugUR study by age groups and sex

A box represents the lower (25%) and upper (75%) quartiles with median as a horizontal line within the box. Y-axis shows values on a log10-based scale. hsTnT: high-sensitivity troponin T.

Figure 2. Proportion below and above a high-sensitivity troponin T rule-out cut-off value of 14 ng/l in different AugUR subgroups.

The proportion of negatives according to the rule-out cut-off value of 14ng/l, who are correctly identified as not having acute myocardial infarction, decreases with sex, age and renal function (blue boxes), whilst the rate of false positives increases (orange boxes). Grey boxes represent the commonly accepted false positive rate of 1%.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. hsTnT: high-sensitivity troponin T. Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m^2). Subcohort II: additionally free of diabetes and obesity (body-mass index <30 kg/m^2) with a blood pressure <160/100mmHg at study visit. Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

Figure 3. Determinants of elevated high-sensitivity troponin T (>14ng/l)

Odds ratio estimates for high-sensitivity troponinT > 14 ng/l. Simple logistic regression without adjustment and after adjustment for age and sex. Presented are the OR and 95% CI. Dashed line indicates OR=1.

$eGFR_{crea}$ glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m^2. LV: left ventricular. Elevated LV filling pressure: E/e' >14.

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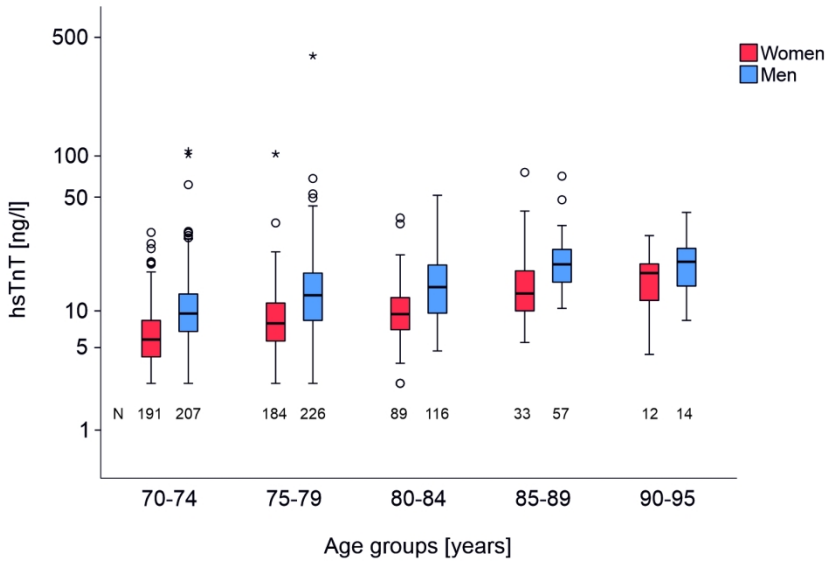

Figure 1. Values of high-sensitivity troponin T in 1,129 participants of the AugUR study by age groups and sex

A box represents the lower (25%) and upper (75%) quartiles with median as a horizontal line within the box. Y-axis shows values on a log10-based scale. hsTnT: high-sensitivity troponin T.

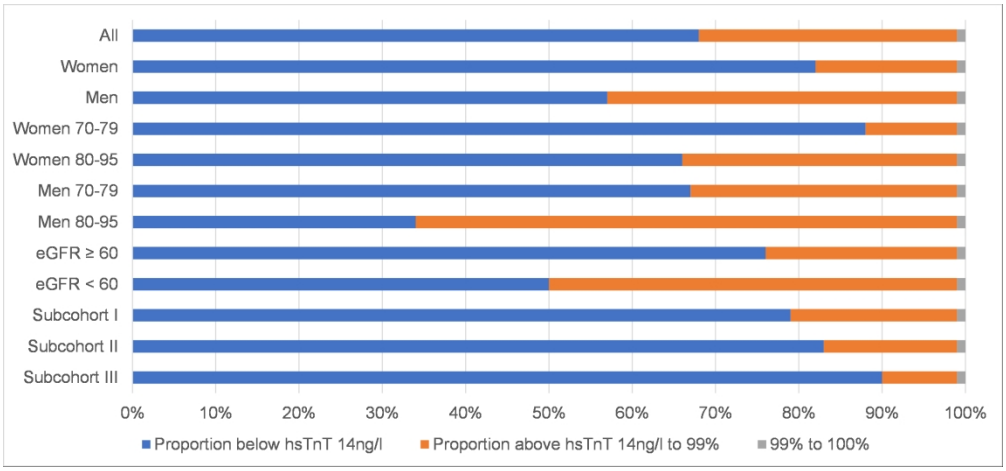

Figure 2. Proportion below and above a high-sensitivity troponin T rule-out cut-off value of 14 ng/l in different AugUR subgroups.

The proportion of negatives according to the rule-out cut-off value of 14ng/l, who are correctly identified as not having acute myocardial infarction, decreases with sex, age and renal function (blue boxes), whilst the rate of false positives increases (orange boxes). Grey boxes represent the commonly accepted false positive rate of 1%.

eGFRcrea glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m2. hsTnT: high-sensitivity troponin T. Subcohort I: subjects free of clinical coronary artery disease and heart failure with normal renal function (eGFR≥60ml/min/1.73m2). Subcohort II: additionally free of diabetes and obesity (body-mass index <30 kg/m2) with a blood pressure <160/100mmHg at study visit. Subcohort III: as subcohort II, additionally in regular heart rhythm, free of left ventricular hypertrophy, of elevated left ventricular filling pressure (E/e' > 14) and of left ventricular systolic dysfunction (EF < 50%).

641x296mm (118 x 118 DPI)

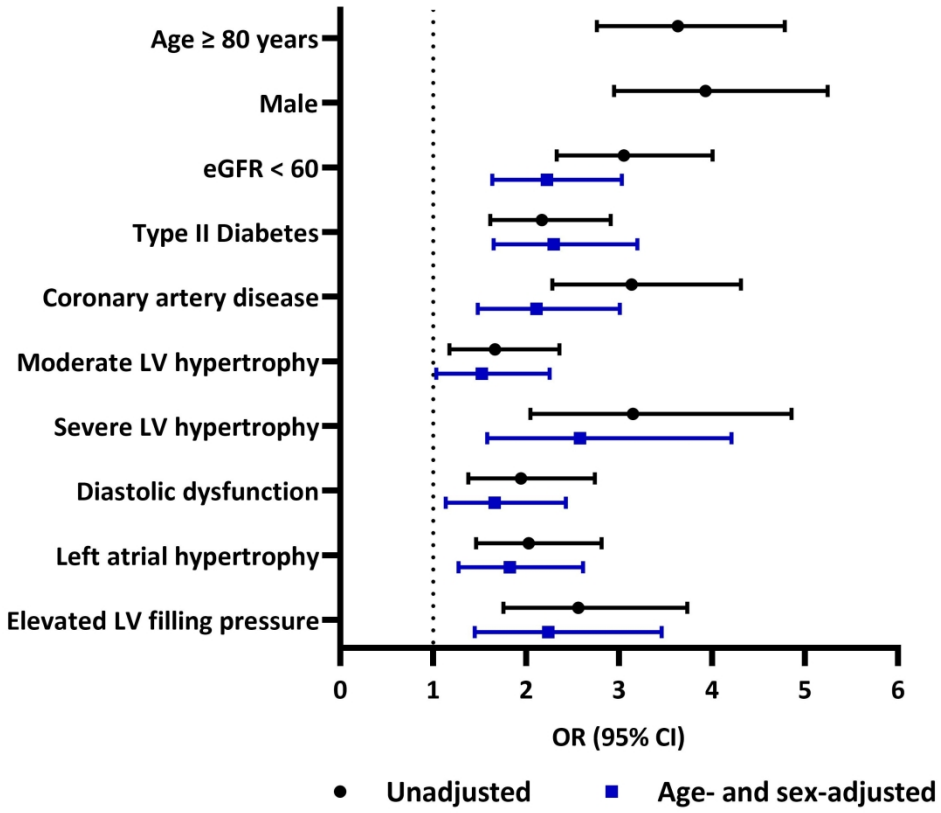

Figure 3. Determinants of elevated high-sensitivity troponin T (>14ng/l)
Odds ratio estimates for high-sensitivity troponinT > 14 ng/l. Simple logistic regression without adjustment and after adjustment for age and sex. Presented are the OR and 95% CI. Dashed line indicates OR=1. eGFRcrea glomerular filtration rate estimated from serum creatinine in ml/min per 1.73m2. LV: left ventricular. Elevated LV filling pressure: E/e' >14.

125x110mm (600 x 600 DPI)

Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectionalreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	#3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	#4	Present key elements of study design early in the paper	5
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5

Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants.	5
	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6-7
Bias	#9	Describe any efforts to address potential sources of bias	22
Study size	#10	Explain how the study size was arrived at	5 and 8
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	7 and 14-15
Statistical methods	#12c	Explain how missing data were addressed	n/a
Statistical methods	#12d	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	#12e	Describe any sensitivity analyses	7 and 14-15
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	8
Participants	#13b	Give reasons for non-participation at each stage	5 and 8

Participants	#13c	Consider use of a flow diagram	n/a
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	8
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	8 and 12
Outcome data	#15	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	6-7
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 3 + caption
Main results	#16b	Report category boundaries when continuous variables were categorized	10,11,13,17, Fig3+caption
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	7, 9-17
Discussion			
Key results	#18	Summarise key results with reference to study objectives	18
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	22
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	18-22
Generalisability	#21	Discuss the generalisability (external validity) of the study results	18-22
Other Information			

Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

Notes:

- 12b: 7 and 14-15
- 12e: 7 and 14-15
- 16a: Figure 3 + caption
- 16b: 10,11,13,17, Fig3+caption This checklist was completed on 02. April 2021 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai